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Einfluss chemisch definierter Stoffe auf die Entwicklung keimender Samen und auf Tumoren

Von HANS VON EULER

Der Befund, dass die Keimblätter von Gerste und anderer grüner Pflanzen sich nach der Keimung der Samen in Streptomycinlösungen farblos und Katalase-arm entwickeln, hat veranlasst, andere ähnlich wirkende Stoffe und dann auch weitere oxen der Chlorophyllbildung aufzusuchen. Das Studium der chemischen Reaktionen, welche die Bildung des Chlorophylls in Streptomycin-behandelten keimenden Samen hemmen, erschien in Rücksicht auf chlorophylldefekte Mutanten, zunächst der chlorophylldefekten Gersten-Mutanten vom Albina-Typus von wesentlichem Interesse.

Aus den früheren Ergebnissen dieses Institutes über diese chlorophylldefekten Mutanten und über den Einfluss chemischer Stoffe auf die Chlorophyllbildung bei der Keimung seien hier nur die folgenden hervorgehoben:

1. Die Hemmung der Chlorophyllbildung in Mutanten vom Albina-Typus (nicht von Xantha, Virescens, Chlorina usw.) ist von einer starken Erniedrigung des Katalase-Gehaltes begleitet. Als gemeinsame Ursache dieser beiden Wirkungen ziehen wir erster Linie das gemeinsame Ausgangsmaterial *Protoporphyrin* in Betracht. Wir finden bei der Mendel-Spaltung der Albina-Mutanten in den grünen Keimblättern 3-mal so hohe Katalase-Wirkung als in den weissen Blättern. Damit war zum erstenmal ein Zusammenhang einer speziellen quantitativ messbaren Enzymwirkung mit einer Mendelspaltung nachgewiesen worden.¹

2. Behandlung der Samen mit Streptomycin (STRM) hemmt die Bildung von Chlorophyll in den Keimblättern, beeinflusst eine Vorstufe des Chlorophylls, erzeugt also, in geeigneter Menge angewandt, weisse Keimblätter. Sie erniedrigt dagegen nicht die Menge des schon gebildeten Chlorophylls.²

3. Behandlung der quellenden Samen mit sehr kleinen STRM-Mengen verursacht nicht eine gleichmässige Erniedrigung des Chlorophyllgehaltes der Keimblätter, der Weise, dass hellgrüne Blätter entstehen, vielmehr ist in diesen Fällen der obere Teil (Spitze) der Keimblätter grün, während der untere Teil farblos bleibt. (siehe Arkiv f. Kemi, Min. Geol. 25 A, nr 17 (1947).

¹ EULER u. H. NILSSON, Arkiv f. Kemi, Min. Geol. 10 B, nr 6 (1929). — EULER, Festschr. Akad. Wissensch. Göttingen 1951, 123. — EULER, Hereditas 13, 61 (1929). — EULER, A. FORSSBERG, L. RUNEHJELM u. H. HELLSTRÖM, Z. Abstammungslehre 59, 131 (1931). — EULER, D. BURSTRÖM u. H. HELLSTRÖM, Hereditas 18, 225 (1933).

² EULER, Kem. Arb. Ny följd 9, II (1947). — EULER u. M. JAARMA, Arkiv f. Kem. Min. Geol. 25 A, nr. 7 (1947). — EULER, Arkiv f. Kem. Min. Geol. 25 A, nr. 17 (1947). — EULER, Arkiv f. Kem. Min. Geol. 26 A, nr. 6 (1948).

4. STRM fällt Nukleinsäuren, Nukleoproteine und Nukleoproteide.¹

5. Chlorophyll-Hemmungen ähnlicher, aber nicht ganz übereinstimmender Art wie mit STRM wurden mit den Tetracyclinen Aureomycin, Achromycin und Terramycin erhalten.² Dieselben zeigen, wie das STRM, auch eine toxische Wirkung auf die Wurzeln (Klaubenbildung).³

Im Anschluss an unsere Versuche mit antibiotischen Stoffen haben wir nun eine Reihe von cytostatischen Stoffen bezüglich Chlorophyll-Hemmung und Entwicklungshemmung untersucht.⁴

Neue Versuche

Streptomycin- und Guanidin-Derivate

Eine geringe Antichlorophyll-Wirkung fanden wir bei *Streptidin*⁵, dem Guanidin-haltigen Spaltprodukt des Streptomycins, das auch Ribonukleinsäure fällt. Viel schwächer (kaum merkbar) hemmen Dekamethylen-diguanid und Dimethylen-diguanid, die Ribonukleat aus 0,1 %iger Lösung fällen.⁶ Eine andere Diguanido-Verbindung, nämlich das Diguanido-butan Arcain hat sich beim Erbsen-Test als stark wachstumshemmend und toxisch (Klaubenform der Wurzeln bei Gerste) erwiesen. Dagegen fanden wir mit Guanidin selbst sowie mit der von uns dargestellten Guanidin-Redukton-Verbindung nur bei *Lepidium sativum* Wirkung auf Keimung und Wurzel-Entwicklung (vgl. Arkiv 25 A, nr 17).

Die durch die gleichzeitige Abnahme des Chlorophyllgehaltes und der Katalase-Wirkung veranlasste Hypothese, dass diesen Effekten eine Hemmung der Protoporphyrin-Bildung zugrunde liegt, legt den Schluss nahe, dass STRM auch die Hämin-Bildung beeinträchtigt. Diesbezügliche Versuche haben wir wiederholt angestellt, und dabei auch die Neubildung roter Blutzellen berücksichtigt. Tatsächlich haben wir mehrmals, aber nicht regelmässig, eine Erhöhung der vitalfärbbaren roten Blutzellen beobachtet.

Über die besonderen Chlorophyllhemmungen beim Erbsen-Test siehe EULER, Z. physiol. Chem. 295, 411 (1953).

N-Oxyd-Lost ist eine der wirksamsten, wenn nicht die wirksamste der tumor-hemmenden Stoffe⁷. Wir konnten die Wirksamkeit dieses Stoffes durch eigene Versuche mit Yoshida-Ascites-Sarkom-Ratten bestätigen. Hier seien nur einige unserer

¹ EULER, M. BRACCO u. L. HELLER, C. r. Acad. Sci. (Paris) 227, 16 (1948). — M. BRACCO u. EULER, Kem. Arb. N. F. 10, II (1948). — R. GIULIANO, M. L. STEIN u. EULER, Arkiv Kemi 6, nr 53 (1954).

² EULER u. M. L. STEIN, Experientia 11, 108 (1955).

³ Bei Vorbehandlung mit anderen Stoffen, z. B. Salicylsäure-cholin-hydrazid und Dinicotinyl-acetyl-sulfanilsäure-hydrazid (EULER u. HASSELQUIST, Z. physiol. Chem. 288 und 295) tritt ein allmählicher Übergang zu hellgrünen Blättern ein.

⁴ Die Chlorophylldefekte in den von uns früher studierten Albina-Mutanten stehen mit vielen, voneinander unabhängigen Genen in Zusammenhang. Man kann nicht mit Bestimmtheit sagen, inwieweit die Chlorophyll-Hemmungen in den chlorophylldefekten Mutanten, in Panachüren u. a. m. und in den mit STRM behandelten Pflanzen wesengleich sind.

⁵ EULER u. L. HELLER, Arkiv Kemi, Min. Geol. 26 A, nr. 14 (1948). — Bez. der Bindung von STRM an Nukleinsäuren siehe auch DISCALUSY und EULER, Arkiv Kemi, Min. Geol. 25 A, nr 16 (1947).

⁶ EULER u. L. HELLER, Arkiv Kemi 1, nr. 35 (1949).

⁷ Zur Verwendung kam ein Präparat der Asta-Werke; Herrn Prof. Dr. BROCK BRACKWEDE spreche ich auch hier meinen besten Dank für die Überlassung des Präparates aus.

tumor-Versuche, die mit BETH v. EULER ausführlicher an anderer Stelle mitgeteilt werden, angegeben:

Versuchsreihe A. In unsere weissen norwegischen Ratten wurde mit wenigen Minuten Zwischenraum injiziert: 250 γ N-Oxyd-Lost und 0,3 ml Ascites, enthaltend 200 000 tumor-Zellen/mm³. Kontroll-Versuche ohne Lost.

In 3 Versuchen wurde der Tod der Ratten, der in den Kontroll-Versuchen nach 5 Tagen eintrat, auf 9 Tage nach der Injektion verzögert.

Versuchs-Reihe B. In zwei Ratten wurden je 0,2 ml Ascites *intramuskular* in den Oberschenkel injiziert, mit dem Erfolg, dass sich im Schenkel in 5 Tagen ein infiltrierender Tumor bildete. An diesem 5. Tage nach der Ascites-Injektion wurden 500 γ N-Oxyd-Lost, am folgenden Tag weitere 1000 γ und am darauf folgenden Tag (dem 7. nach der Ascites-Injektion) nochmals 1000 γ N-Oxyd-Lost injiziert. Am 13. Tag (also 6 Tage nach der letzten Lost-Injektion) war der Oberschenkel-Tumor vollständig verschwunden: das Tier *erschien vollständig geheilt*.

Eine dritte Ratte erhielt, wie die beiden anderen, 0,2 ml Ascites, und am 6. Tag nach dieser Injektion, als sich ein grosser Tumor an der Injektionsstelle gebildet hatte, 1000 γ Lost. Nach 3 weiteren Tagen wurden, da der Tumor noch nicht zurückgebildet war, noch 500 γ Lost injiziert, und am 13. Tag nach der Ascites-Injektion war das Tier *anscheinend geheilt*.

Besonders bemerkenswert ist, dass dieses Tier bald nacher, 6 Tage nach der letzten Lost-Injektion, nach einer intraperitonealen Ascites-Injektion diesen nicht weiter entwickelte. Sofern nicht noch Lost im Tier vorhanden und verbreitet war, muss das Ausbleiben der Tumor-Entwicklung auf eine entstandene *Immunität* zurückgeführt werden. Indessen trat dieser Immunitäts-Effekt keineswegs regelmässig ein.

In weiteren Versuchen waren die durch Ascites hervorgerufenen intramuskularen Tumoren durch 750 γ des N-Oxyd-Lost zum Verschwinden gebracht worden.

Ausser diesen Fällen von Tumor-Hemmung nach intramuskularer Injektion von Ascites sei nur noch erwähnt, dass Ascites-Tumoren, die nach *subcutaner* Injektion von Ascites entstanden sind¹, durch die — allerdings hohen — Dosen von 2,5 mg N-Oxyd-Lost *geheilt wurden*.

a) Keimungs-Versuche mit N-Oxyd-Lost

Zu Keimungsversuchen an Gerste wurden in Vorversuchen die geeigneten Lost-Konzentrationen der Keimungslösungen sowie das geeignete Verhältnis zwischen Samenmenge und Volumen der Keimungslösung ermittelt. Die wegen zu hoher Verdünnung unwirksamen und andererseits die zu hoch konzentrierten und deshalb zu stark toxischen Lösungen wurden ausgeschieden und zu den definitiven Versuchen kamen folgende Zusammensetzungen zur Anwendung:

5 g Samen in 50 ml Lösung,
enthaltend 20–80 mg N-Oxyd-Lost.

Man liess also 5 g Gerstensamen (Hertha-Gerste, Weibullsholm) 8 Stunden in 100 ml Wasser (unter Zusatz von 0,05 % H₂O₂) und dann 20 Stunden in 50 ml Lösung

¹ Die durch intramuskuläre bzw. subcutane Injektionen von Ascites erzeugten Tumoren sind anscheinend von den in der Bauchhöhle entstehenden festen Yoshida-Ascites-Tumoren durchaus verschieden und haben die Konsistenz und das Aussehen wie etwa Jentschentumoren. Sie werden hier durch Transplantation weiter gezüchtet.

von 50 mg N-Oxyd-Lost bei Raum-Temp. 18° stehen und überführte dann alles in Glas-Schalen, in denen die Samen 6 Tage zwischen Filtrierpapier und dann unbedeckt keimten.

Schon an 4. Keimungstag machte sich der Unterschied zwischen Lost-Versuch und Wasser-Kontroll-Versuch bemerkbar: Die Keimlinge in der Lost-Lösung waren kürzer als 1 mm, diejenigen in Wasser im Mittel 3,5 mm. Während die normalen Keimblätter nach 10 Tagen 35 mm lang waren, blieben die in der Lost-Lösung entwickelten weit zurück, mittl. Länge 15 mm, nach 18 Tagen 25 mm. Diese Länge wurde kaum mehr überschritten und betrug nach 20 Tagen 25–27 mm. Die kleinen Keimblätter waren grün. Die Wurzeln bildeten sich klauenförmig aus.

b) Keimungs-Versuche mit Benzpyren

Folgende orientierende Versuche über Keimungshemmungen seien mitgeteilt:

Gersten-Samen-Test. Hertha-Gerste, Weibullsholm. 5 g Samen wurden nach 8stündiger Quellung in Wasser in 50 ml Suspension von 1 mg *Benzpyren* in 2%iger Na-Cholat-Lösung übergeführt. Nach 22 Tagen wurden die Samen mit der Suspension zwischen Filtrierpapier in Schalen gelegt. Dann wurde die Filtrierpapier-Decke entfernt und dadurch die Samen dem diffusen Tageslicht ausgesetzt.

Nach 10 Tagen waren nur 4 % der Samen gekeimt, und zwar betrug die mittlere Länge der Keimlinge etwa 2 mm; bei etwa der Hälfte dieser Samen wurde Wurzelbildung beobachtet. Nach weiteren 8 Tagen betrug die Länge der Keimlinge 2–5 mm; die 1–3 mm kurzen Wurzeln waren dick und gekrümmt.

Auch das zur Lösung bzw. Suspension des Benzpyrens verwendete Na-Cholat (Natrium cholum, Schering) war an obigem Effekt wesentlich beteiligt. Zum Vergleich wurde folgender Kontroll-Versuch angestellt:

In 2%iger Na-cholatlösung (auf pH 6 eingestellt) keimten nach im ganzen 12 Vorbehandlungstagen 7 % der Samen, und zwar erreichten die Keimlinge eine mittlere Länge von 2 mm, während die Wurzellänge rund 5 mm betrug. Nach weiteren 10 Tagen waren die Keimlinge 5–10 mm lang. Es zeigt sich also, dass das Benzpyren trotz der ungünstigen Löslichkeitsverhältnisse die Keimung der Gerstensamen mehr gehemmt hat als das Na-Cholat. Exakte quantitative Ergebnisse lassen sich aber mit den Suspensionen wegen der Unregelmässigkeit der Berührung der suspendierten Substanz mit den Samen nicht feststellen.

Deswegen wird auch auf die nähere Beschreibung der Keimungs-Versuche mit Dimethylamido-azobenzol verzichtet. Mit Yoshida-Ascites Ratten wurden bei Versuchsanordnungen, analog den unten bez. Benzpyren beschriebenen, durch Buttergelbdosen von 5 mg in 4 Injektionen sichere Verzögerungen des Tumor-Wachstums erzielt, so dass die Lebensdauer in 8 Tierversuchen von 4,5 Tagen auf 10–12 Tagen stieg. Wie nochmals betont werden soll, kam es uns hier nur darauf an, den Hemmungseffekt durch 2 typische kancerogene Substanzen nachzuweisen.

Hemmung der Chlorophyllbildung durch Keimung bei Temperaturen zwischen 40 und 50°. Die bisher erhaltenen Resultate waren so unregelmässig, dass nur angegeben werden kann, dass in 2 von 5 Versuchsreihen nach 22 Minuten langer Erwärmung der vorher in Wasser bei pH 5,5 gequollenen Samen Blätter mit geringem Chlorophyllgehalt entstanden sind.

Benzpyren im Tumor- und Gersten-Versuch

Benzpyren-Injektionen intraperitoneal gleichzeitig mit Yoshida-Ratten-Ascites (0,3 ml).

5 mg Benzpyren-Injektion von 1 mg 1 %iger Na-Cholat-Lösung (pH 6). 4 Injektionen zu je 1,25 mg Benzpyren per Tag. 10 Versuchstiere. 7 Kontrollen. Lebensdauer d. Kontrolltiere 5–5,5 Tage. Lebensdauer der Versuchstiere:

- 3 tot nach 5–6 Tagen (180 γ)
- 3 tot nach 8–9 Tagen (3 Injektionen zu je 1,25 mg)
- 2 tot nach 13 Tagen (4 Injektionen zu je 1,25 mg)
- 2 leben nach 15 Tagen (7 Injektionen zu je 1,25 mg).

Unter den Stoffen, welche der Untersuchung von EULER u. PERJE¹ zufolge den Entwicklungsgrad keimender Gerste beeinflussen, haben uns die Urethane wiederholt beschäftigt. Schon durch Vorbehandlung mit 0,005 % Phenylurethan entstand in keimenden Gerstensamen eine Verzögerung des Coleoptilwachstums, und durch Behandlung von Gersten- und Krasse-Samen mit Äthylcarbammat werden Katalase- und andere Enzymwirkungen gehemmt.

Unsere bis jetzt mit Phenylurethan erhaltenen schwachen Effekte wurden deutlich verstärkt durch Vorbehandlung der 24 Stunden in Wasser gequollenen Samen mit Ascorbinsäure – Hydroperoxyd, und zwar, wie wir annehmen, durch Erhöhung der Permeabilität der Zellmembranen. Wir werden auf diese Versuche bald ausführlich zurückkommen.

Andererseits haben HADDOW und SEXTON² mit Phenylurethan (Äthylphenylcarbammat) und auch mit Äthylcarbammat deutliche Verzögerungen des Wachstums von spontanen Mammacarcinomen bei Mäusen hervorgerufen. Sowohl diesen Effekt als die Beeinflussung der Samenkeimung kann man auf eine Störung des enzymatischen Purinstoffwechsels zurückführen.

Von besonderer Bedeutung ist die durch HADDOW und durch K. H. BAUER³ begründete Feststellung, dass mehrere Kohlenwasserstoffe, deren cancerogene Wirkung auf Muskelgewebe normaler Ratten von KENNAWAY erkannt worden war, auf Tumorzellen einen hemmenden Einfluss ausüben, und die Aufklärung der Tatsache, dass auch andere Stoffe, welche an normalen Zellen eine cancerogene Mutation hervorrufen können, die Entwicklung canceröser Zellen hindern, dürfte ein wichtiges Problem der biochemischen Tumorforschung bilden.

Können Mutationen ähnlicher Art — etwa durch Chromosomen-Verkettung in den Zellkernen — bei der Samenkeimung auftreten?

Bei der Fortsetzung unserer Studien über die Entwicklungs-Hemmungen keimender Samen wurde versucht, einige an der Keimung beteiligte Wirkungszentren zu inaktivieren, und wir haben deswegen Gerstensamen in Gegenwart von Benzpyren sowie von Dimethylaminoazobenzol keimen lassen.

Die Unlöslichkeit des Benzpyrens und des Azofarbstoffes in Wasser machte es notwendig — um den Kontakt dieser Substanzen mit dem keimenden Material herzustellen —, Na-Cholat zu einer Suspension der beiden cancerogenen Stoffe zuzusetzen.

Keimungshemmung an Gerste (Siegergerste, Svalöv) wurde früher¹ durch Coffein

¹ EULER u. A.-M. PERJE, Arkiv Kemi, Min. Geol. 20 A, nr 2 (1945).

² A. HADDOW u. W. A. SEXTON, Nature 157, 500 (1946).

³ K. H. BAUER, Das Krebsproblem. Springer, Berlin, p. 589 u. ff. (1949).

in 0,08 %iger wässriger Lösung erreicht (besonders wurde Hemmung des Coleoptilwachstums hervorgehoben). Später hat sich gezeigt (unpublizierte Versuche von RÖNNESTAM-SÄBERG und GERNOW), dass andere Purinderivate, z. B. Amino-cofein, Hypoxanthin und Theophyllin stärker hemmen. Genaue Untersuchungen werden, besonders in letzterem Fall (Theophyllin), durch die Schwerlöslichkeit erschwert. Auffallend ist bei den Theophyllin-Versuchen, dass in etwa 10 % der in Theophyllinlösungen gequollenen Samen die Keimlinge in 20–25 Tagen Längen bis 30 mm erreichen, während die Wurzeln sich kaum entwickeln (dicke, 3–4 mm lange, gekrümmte Wurzeln).

Schliesslich sei noch die Tumorchemmung durch ein Chinon erwähnt, das anlässlich einer Redukton-Untersuchung aus 4-Phenyl-2,3-diketo-butyrolakton dargestellt wurde (H. HASSELQUIST). Auch diese Substanz ist sehr schwerlöslich in Wasser, aber löslich in verdünntem Alkali bei pH 7,5. Sie wurde im Verhältnis 2 mg/ml suspendiert. Von der Suspension wurde 0,5 ml mit 0,3 ml Ascites gemischt, 14 Stunden im Eisschrank bei 2–3° stehen gelassen und dann 0,3 ml der Mischung injiziert.

Kontrollversuch mit Kochsalzlösung statt Suspension. Lebensdauer 4–5 Tage. In 3 Versuchen mit unserem Chinon I zeigte die Sektion nach 7 Tagen keinen Ascites und keinen festen Tumor.

Stockholm, Universität, Vitamin-Inst. u. Inst. f. organ. chem. Forschung.

Tryckt den 15 november 1955

Uppsala 1955. Almqvist & Wiksells Boktryckeri AB

Identification and determination of mercury in biological materials

By HANS WANNTORP and ARNE DYFVERMAN

With 10 figures in the text

Nowadays mercury and mercurial compounds are not only used in Medicine but also to a great extent as pesticides in fruit growing, as baits for seed, etc. Through the extensive use of such agents, especially by laymen, cases of poisoning of man and domestic animals are not very infrequent. As far as domestic animals are concerned mercurial poisonings generally are due to the use of seed baited with mercury in feed. In order to be able to identify and estimate mercury in body tissues and excretions in cases of suspected poisoning a method of analysis is required which is highly sensitive and not too complicated.

In the quantitative estimation of mercury, colorimetric analysis using dithizone appears to be the most suitable method. Dithizone was introduced and tested out on a number of heavy metals by H. FISCHER and co-workers (1, 2). Dithizone is used dissolved in an organic solvent, usually chloroform or carbon tetrachloride. Such a solution is intensely green in colour, but dithizone compounds with metals produce other colours on solution. By measurement of the colour intensity of the dithizone solution at a suitable wave-length after agitation with a test solution under known conditions certain metals which produce complex dithizone compounds can be estimated quantitatively.

This method was first utilised for the determination of mercury by W. O. WINKLER (3) and by H. FISCHER and G. LEOPOLDI (4). Later a large number of investigations into this method were performed and many modifications have been proposed. These have shown that the dithizone method can be widely applied but that there are complications involved in performing the test which are difficult to overcome. The complications are, *inter alia*, due to the following circumstances:

- 1) The high sensitivity usually sought after necessitates a very high degree of cleanness in the utensils and reagents.

- 2) Dithizone is easily oxidised by air and oxidising agents and therefore cannot be obtained commercially in a pure form. Nor does dithizone in a purified solution exist only in a pure form, and it cannot be stored unchanged.

- 3) Certain anions, such as halogen, thiocyanate and thiosulfate ions which form complexes with mercury, or which on combustion are converted into oxidising agents, can, especially in strongly acid solution, upset more or less seriously the determination of mercury.

4) Dithizone forms in addition to mercury complexes also complex compounds with several other metals (Mn^{II} , Fe^{II} , Co^{II} , Ni^{II} , Pd^{II} , Pt^{II} , Cu^{I} , Cu^{II} , Ag^{I} , Au^{I} , Zn^{II} , Cd^{II} , In^{III} , Tl^{I} , Tl^{III} , Sn^{II} , Pb^{II} , Bi^{III}). Therefore it is necessary in the determination of mercury either to remove these metals first or to carry out the analysis under such conditions that they do not react with dithizone.

The majority of the metals, which could disturb the determination of mercury are prevented from reacting with dithizone if the extraction is carried out in a mineral acid solution, approximately 0.5–1 N. Noble metals, such as silver, gold, platinum and palladium are, however, also extracted in acid solution and copper is partially extracted. The extraction of mercury with dithizone has previously usually been carried out in acid solution.

It is also possible, through the addition of certain substances or ions which form complex compounds with metals, to prevent them from reacting with dithizone. This is described as "masking" the metals. V. VAŠÁK and V. ŠEDIVÉČ (5) used ethylenediaminetetraacetic acid, or versene, one of Schwarzenbach's complexones, as a masking agent. Versene masks the same metals as diluted mineral acids with at least the same effectivity. In the presence of versene they carry out the extraction in a weakly acid solution at approx. pH 4.6. The use of this compound has some important advantages.

a) In the weakly acid, buffered solution dithizone is more stable and less subject to the effect of oxidising agents. Nitrate ions, for example, have no effect on dithizone in a weakly acid solution, but they have an increasing tendency to oxidise the reagent with a rise in acidity.

b) The anions mentioned above (point 3), which complicate or completely prevent the estimation of mercury with dithizone in strongly acid solution, have considerably less effect in weakly acid solution.

The dithizone-versene method (5) has been tested and modified for the determination of mercury in biological material in the present investigations.

Determination of mercury

Principle

The organic matter is combusted with nitric and sulfuric acid with collection of the condensate in a reflux collector by the method elaborated by A. K. KLEIN (6). This method permits effective combustion without loss of mercury through distillation. After conclusion of the combustion the contents of the flask are partially neutralised with ammonium hydroxide and the nitrous gases are removed with hydroxylamine hydrochloride.

After extraction with a solution of dithizone in carbon tetrachloride the mercurial content of the extract is determined photometrically by the mixed-colour method, based on the determination of mercury dithizonate (HgDz_2) in the presence of an excess of dithizone (Dz). The components can be determined by measurement of the absorbancy at two different wave-lengths. Using carbon tetrachloride as solvent the absorption curve for HgDz_2 has its maximum at 490 m μ , and the corresponding curve for Dz has its most important maximum at 620 m μ and a minimum at 515 m μ (Fig. 1).

Dz is determined at 620 m μ . At this wave-length mercury dithizonate has practically no absorption. On the other hand, the absorption of dithizone interferes in the

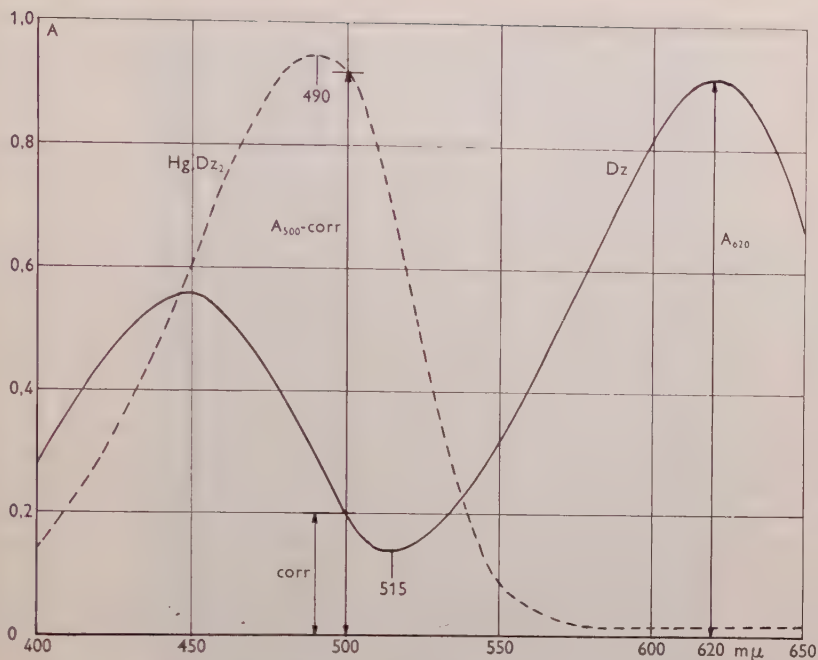


Fig. 1. Absorption curves for dithizone (Dz) and mercury dithizonate (HgDz_2) in carbon tetrachloride.

determination of HgDz_2 . In order to reduce this interference, readings are made at 500 $\text{m}\mu$, somewhat to one side of the absorption maximum for HgDz_2 . Correction for the absorption of dithizone was made in approximately the same way as F. L. HOCH and B. L. VALLEE (7) did in the determination of zinc. The corrected absorbancy was calculated as follows:

$$A_{\text{corr}} = A_{500} - \frac{A_{500}^0}{A_{620}^0} \cdot A_{620}$$

A_{corr} = corrected absorbancy at 500 $\text{m}\mu$.

A_{500} and A_{620} = absorbancies for the extract at 500 and 620 $\text{m}\mu$ respectively per 1 cm cell length read against carbon tetrachloride.

A_{500}^0 and A_{620}^0 = absorbancies for the dithizone solution before extraction.

The mercury content was calculated both from the content of HgDz_2 (method A) and from the reduction in the content of Dz (method B). The values from method A were better related to the real mercury content than from method B. The latter were, however, of value as controls, as disturbances from, for example, oxidising agents or other metals were detectable as a divergence between A and B. The mean of the results from A and B were calculated at first (method C). When it became apparent that the calculation of mean values was not advantageous method C was not employed other than as a comparative method in the investigation of the influence of copper on the analysis.

The best results were obtained if the whole analysis was performed in one day. Spread over two days usually resulted in a 10–20 % loss of mercury. One person can usually finish the analyses of two samples and a blank during one working day if helped with the extraction procedure.

Apparatus and reagents

The digestion apparatus (6) (Fig. 2) consists of a double-necked 500 ml digestion flask (A) provided with a reflux collector (B) in the form of a modified Soxhlet apparatus with a stopcock (C) and a reflux condenser (D). In the second neck of the flask a dropping funnel (E) is placed. The connections are all standard taper 24/40. The tube (F) on the reflux collector is insulated, with for example, asbestos wool.

Separatory funnels. 500 ml separatory funnels with glass stoppers are used.

All glassware, which must be thoroughly cleaned, is washed before use with a dithizone solution for cleaning (see below) and then with water. Taps and stoppers are smeared with vaseline as proposed by KLEIN (8).

Mercury standard. 676.7 mg of HgCl_2 are dissolved in water and the solution diluted to 500 ml. This solution contains 1 mg of Hg/ml.

Diluted mercury standard. 5 ml of mercury standard are diluted to 250 ml. This solution contains 20 μg Hg/ml and is stable for 1–2 weeks in a refrigerator. If more dilute solutions are used they must be prepared when needed.

Nitric acid, concentrated.

Acid mixture. Nitric acid, conc., and sulfuric acid, conc. (1 + 1).

Dilute sulfuric acid, 2 N.

Ammonium hydroxide, conc.

Bromocresol green, 0.5 % in alcohol.

Hydroxylamine hydrochloride.

Carbon tetrachloride.

Dithizone solution. The dithizone is purified largely according to BLAXTER and ALLCROFT (9) as follows: Dithizone (0.1 g) is dissolved in carbon tetrachloride (100 ml). The solution is agitated in a 500 ml separatory funnel 3–4 times with 100 ml of ammonia (1:20). The whole of the ammoniacal extract is filtered and weakly acidified with dilute sulfuric acid, which causes the purified dithizone to precipitate. It is then dissolved by shaking with carbon tetrachloride (200 ml). The dithizone solution is separated from the water phase and diluted with sufficient carbon tetrachloride so that 1 ml of the final solution plus 15 ml of carbon tetrachloride produces an absorbancy of approx. 0.9 at 620 m μ in a 1 cm cell. The solution is stored in a flask with a glass stopper in a refrigerator under a layer of sulfurous acid (50 grams of anhydrous sodium sulfite are dissolved in 2 N sulfuric acid to about 500 ml).

Dithizone solution for washing is prepared by dissolving a small amount of dithizone (approx. 25 mg/litre) in carbon tetrachloride.

Buffer solution. 2 moles of sodium acetate ($\text{NaOAc} \cdot 3 \text{H}_2\text{O}$, 272 g) and 2 moles of glacial acetic acid (120 g) are dissolved in water and the solution diluted to 1 litre (pH about 4.6).

Versene solution. Ethylenediaminetetraacetic acid, disodiumsalt (37.2 g), is dissolved in water and then diluted to 1 litre (0.1 molar solution).

Ordinary distilled water and analytical reagents are used.

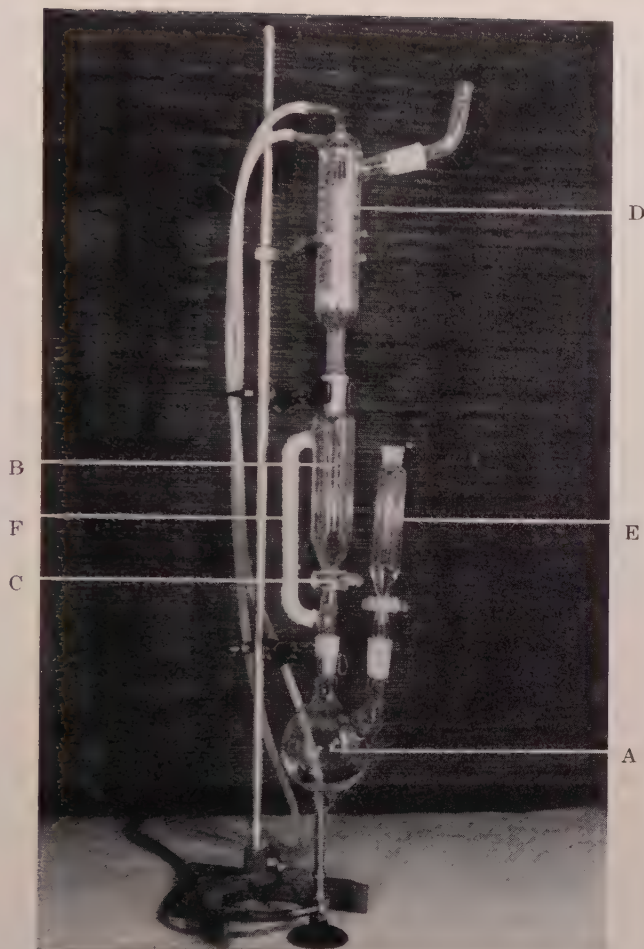


Fig. 2. Digestion apparatus according to A. K. KLEIN (6).

Procedure

Combustion. The material (25–30 g of tissue or 15 g of seed) is weighed in the digestion flask (Fig. 2), then water (10 and 25 ml. respectively) and boiling stones are added. The combustion apparatus is connected with the stopcock (C) open and with cooling water circulating. 20 ml of nitric acid are added through the dropping funnel and the flask heated in a gas flame. The contents are allowed to boil slowly for a few minutes. When the froth has disappeared heating is stopped and after a little while acid mixture (20 ml) is added through the dropping funnel, heating recommenced and the mixture allowed to boil slowly. After a few minutes the stopcock (C) is closed so that the condensate is collected in the reflux collector while the contents of the digestion flask are concentrated. When the contents of the flask begin to darken heating is stopped, a few ml of nitric acid slowly added through the dropping funnel and heating recommenced. This is repeated each time the contents of the flask

become dark. When sulfuric acid fumes are visible in the flask the flame is reduced to minimum. The sulfuric acid fumes must not rise higher than the lower part of the condenser. After 5 minutes heating is stopped.

When the contents of the flask have cooled somewhat the stopcock (C) is opened and the condensate is carefully returned to the digestion flask. The solution is boiled slowly for 5 minutes to rinse down the remaining fatty substances in the condenser and reflux collector. With the stopcock (C) closed the flask is heated and the condensate once again collected in (B). When the contents of the flask darken, nitric acid is added as before. Boiling is continued until sulfuric acid fumes are again visible. After this minimal heating is continued for 15 minutes.

When the flask has cooled a little the condensate is once again carefully released into it. After cooling to below 40°C the flask is removed and the contents emptied into a 500 ml Erlenmeyer flask with standard socket. The digestion flask is rinsed with 10 ml of water which is also emptied into the Erlenmeyer flask. The digestion flask is filled with 25 ml of water and replaced in the combustion apparatus, with the stopcock (C) open and the cooling water shut off and the flask is heated until fumes begin to appear above the condenser. When the digestion flask has cooled somewhat its contents are transferred to the Erlenmeyer flask. The digestion flask is rinsed with a further 10 ml of water.

It is important that combustion is carried out fairly slowly. Rapid boiling results in incomplete combustion and can lead to great losses of mercury vapour through the condenser.

A blank sample of a pure organic substance such as sucrose (about 1 g) is processed simultaneously with the same quantities of nitric and sulfuric acid as used for the sample.

Neutralisation. The neutralisation in the Erlenmeyer flask is carried out with ammonia in a cooling water bath, very carefully to begin with. When the acid has been partially neutralised, 8 drops of bromocresol green are added. The addition of ammonia is continued but eventually only dropwise until the indicator begins to change to green. As overneutralisation can result in loss of mercury the pH should not exceed 5.

To remove nitrite, hydroxylamine hydrochloride (1 g) is added and the flask heated, with an air condenser in place, until the solution just begins to boil, and then it is cooled to room temperature. If one drop of the solution is placed on a strip of potassium iodide starch paper which is then moistened with dilute sulfuric acid, the strip will turn blue if nitrite remains. If this is the case the hydroxylamine hydrochloride treatment is repeated. (In the presence of large quantities of certain metals, e.g. copper salts, a blue colour also occurs.)

By the dropwise addition of ammonia the pH of the liquid is then adjusted using pH indicator paper to 4.5 to 5. Overneutralisation, which should be avoided, is immediately counteracted by the addition of dilute sulfuric acid. After further cooling, non-combusted fatty substances separate in a more or less solid state. The fluid is filtered through a 15 cm filter paper (Munktell No. 00) into a 500 ml separatory funnel, and flask and filter washed with a total of 40 ml water.

Extraction. To the filtrate are added buffer solution (10 ml) and versene solution (25 ml), and the separatory funnel is shaken with carbon tetrachloride (25 ml), which is then removed and discarded. The aqueous solution is now saturated with CCl_4 . As a filter a little cotton-wool is inserted into the stem and pushed up against the stopcock.

If the mercury content is unknown, 15.0 ml of carbon tetrachloride and 1.0 ml of dithizone solution are mixed. The absorbancy is measured spectrophotometrically in a 1 cm cell at wave-lengths 500 and 620 m μ against carbon tetrachloride in a similar cell (A_{500}^0 and A_{620}^0).

The dithizone solution, also that in the cell, is poured as quantitatively as possible into the separatory funnel, which is then agitated vigorously for 1 minute. If the carbon tetrachloride phase is more or less green after separation of the phases, a couple of ml of the carbon tetrachloride phase are drawn off through the filter and returned to the separatory funnel. The absorbancy of the carbon tetrachloride phase is then determined at 500 and 620 m μ as described above.

The solution in the photometric cell is returned to the separatory funnel which is shaken for additionally 1 minute. When the layers have separated a couple of ml of the organic solution are drawn off as before through the cotton-wool filter and returned to the separatory funnel. The absorbancy of the carbon tetrachloride phase is then determined at 500 and 620 m μ . The agitations for 1-minute periods are repeated until the absorbancy at 500 m μ has become almost constant—usually after 1 to 3 periods. In certain cases, e.g. in the presence of copper (see below), the absorbancy at 500 m μ does not, however, become constant.

If the organic phase after the first or any of the following agitations becomes orange-yellow in colour, all the dithizone has been used up in the formation of mercury dithizonate. Additional carbon tetrachloride and dithizone solution are then added in the same proportions as before and agitation is continued until a mixed colour is obtained. The total additions of carbon tetrachloride and dithizone solution are noted for calculation of the volume (V) of the organic phase.

If the mercury content is considered to be greater than 30 μ g, larger quantities of carbon tetrachloride and dithizone solution are mixed at the beginning—for mercury contents of up to 90 μ g amounts of 50.0 + 3.0 ml are suitable. If the mercury content exceeds 150 to 200 μ g, the filtrate is diluted and an aliquot removed for extraction.

The standard curves. In a separatory funnel water (200 ml), buffer solution (10 ml), versene solution (25 ml) and carbon tetrachloride (25 ml) are agitated for $\frac{1}{2}$ minute. When the layers have separated the carbon tetrachloride is removed and discarded. Carbon tetrachloride (50.0 ml) and dithizone solution (3.0 ml) are mixed and the absorbancy of the fluid determined at 500 and 620 m μ (A_{500}^0 and A_{620}^0). The dithizone solution is transferred quantitatively to the separatory funnel which is then agitated vigorously for $\frac{1}{2}$ to 1 minute. When the layers have separated, removal of the organic phase, replacement of it, etc. are carried out as described above. The absorbancy at 500 and 620 m μ is determined and the contents of the cell returned to the separatory funnel.

Diluted mercury standard, in portions of 1.00 or 2.00 ml, is then added to the separatory funnel. After each addition it is shaken vigorously for $\frac{1}{2}$ to 1 minute. When the layers have separated, a couple of ml of the carbon tetrachloride phase are removed and replaced before the sample for determination of absorbancy is taken. The calculation of the standard curves is illustrated by an example in Table 1. Both the upper rows show the measurements in the dithizone solution before extraction (A_{500}^0 and A_{620}^0). The expressions

$$VA_{\text{corr}} \quad (\text{method A})$$

$$\text{and} \quad VA_{620}^0 - VA_{620} \quad (\text{method B}),$$

Table 1. Calculation of standard curves.

Carbon tetra-chloride ml	Dithi-zone ml	Volume V ml	Total Hg added μg	Agita-tion time min.	Calculated by B			Calculated by A		Note
					A_{620}	$V A_{620}$	$V A_{620}^0 - V A_{620}$	A_{500}	$A_{500} - \frac{A_{500}^0}{A_{620}^0} \times A_{620}$	
50	3	53	/		.811 .812	43.0		.168 .168		Values for A^0
			0	$\frac{1}{2}$	.800	42.4	.6	.174	.008	
			40	"	.545	28.9	14.1	.367	.254	
			80	"	.300	15.9	27.1	.564	.502	
			100	"	.171	9.1	33.9	.668	.633	
			120	"	.050	2.7	40.3	.760	.750	

Table 2. Calculation of results.

Sample	Hg added μg	Carbon tetra-chloride ml	Dithi-zone ml	Volume V ml	Agita-tion time min.	Calculated by B. $k_B = 0.33$				Calculated by A. $k_A = 0.33$			Notes
						A_{620}	$V A_{620}$	$V A_{620}^0 - V A_{620}$	$\frac{V A_{620}^0}{k_B} - \frac{V A_{620}}{\mu\text{g}}$	A_{500}	$A_{500} - \frac{A_{500}^0}{A_{620}^0} \times A_{620}$	$\frac{V A_{500}^0}{k_A} - \frac{V A_{500}}{\mu\text{g}}$	
Swine liver 25 g	60	50	3	53		.795	42.1			.172			Values for A^0
				1		.400	21.2	20.9	63.3	.475	.388	62.3	
				1		.395				.475			
Same liver 25 g	0	15	1	16		.840	13.4			.185			Values for A^0
				1		.735	11.8	1.6	4.8	.228	.066	3.2	
				1		.715				.228			
Mercury, found (difference)								58.5				59.1	

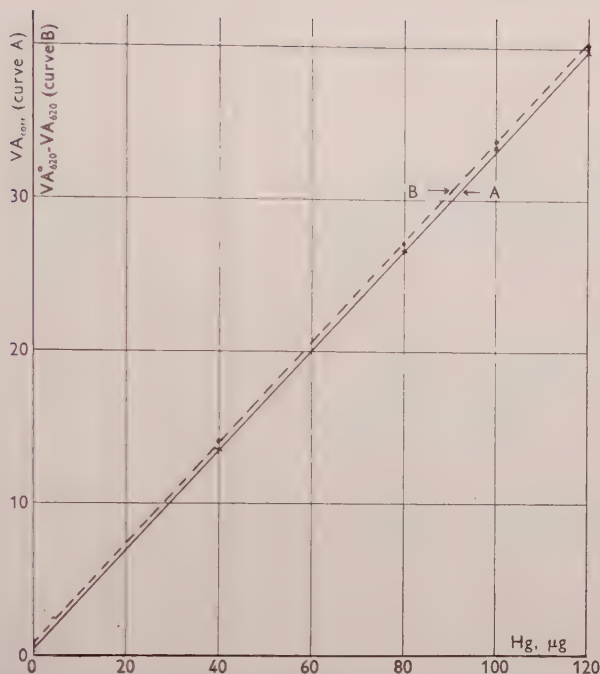


Fig. 3. Standard curves with carbon tetrachloride as solvent. Calculated by method A: $k_A = 0.33$, by method B: $k_B = 0.33$.

where V = volume of the carbon tetrachloride phase in ml, are calculated and plotted against the amount of mercury added in μg (Fig. 3). As the graphs are straight-lined their angular coefficients, k_A and k_B , are used in calculating the results of the analyses. In this case both k_A and k_B are 0.33. Further determinations with carbon tetrachloride as solvent have given the same result.

Calculation of results. The mercury content is calculated by

$$\text{method A: } \frac{VA_{\text{corr}}}{k_A} \mu\text{g mercury}$$

$$\text{method B: } \frac{VA_{620}^0 - VA_{620}}{k_B} \mu\text{g mercury}$$

as illustrated by the example in Table 2. No correction is made for the fact that the standard curves do not pass exactly through origo. The values obtained are reduced by the blank values. In the example the result of calculation according to method A is 59.1 μg (added Hg 60 μg). The control value obtained by calculation according to method B is 58.5 μg and does not indicate the presence of interfering substances.

Chloroform as solvent

At first the mercury was extracted with dithizone (Schering-Kahlbaum) dissolved in chloroform. It appeared, however, that extraction was unsatisfactory if dithizone

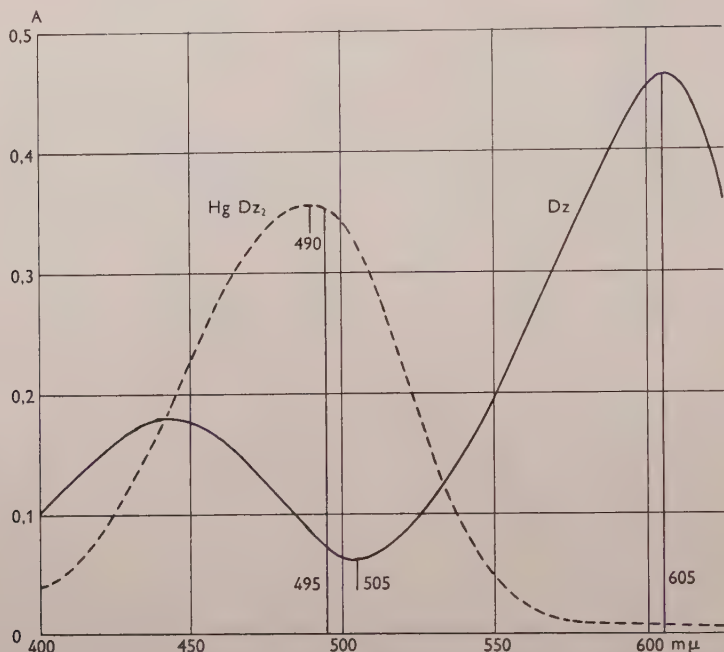


Fig. 4. Absorption curves for dithizone and mercury dithizonate in chloroform.

from other sources was used in a chloroform solution. The disadvantages were difficulty in reaching equilibrium and non-linear standard curves. When Schering-Kahlbaum's dithizone was no longer available, carbon tetrachloride was used as solvent. With carbon tetrachloride the disadvantages mentioned did not appear. The cause of this is as yet unknown to the present authors. Chloroform has otherwise been in general use as a solvent for dithizone, even in the estimation of mercury.

When chloroform was used as solvent the solution was shaken 3 times with 25 ml of chloroform before extraction and the chloroform was discarded. This removes chloroform-soluble colouring matter. Dithizone in chloroform solution was purified with ammonia (1:100) according to BLAXTER and ALLCROFT (9). The absorbancies were read at wave-lengths 495 and 605 mμ in 1 cm photometric cells against chloroform (Fig. 4). Otherwise the procedure and calculations were carried out in an analogous manner as described above.

Fig. 5 shows the standard curves with chloroform as solvent. Many determinations of the slope of these curves with varying volumes of the water and chloroform phases produced almost identical results: $k_A = 0.33$ and $k_B = 0.40$.

Results of determinations. Interference

Results from determinations of mercury in organic material are presented in Tables 3 and 6 (carbon tetrachloride and chloroform, respectively, as solvent for dithizone). The material, muscle, liver or kidney, was analysed with the addition of mercury (10–100 μg). The mercury recovered (after subtraction of the blank) is

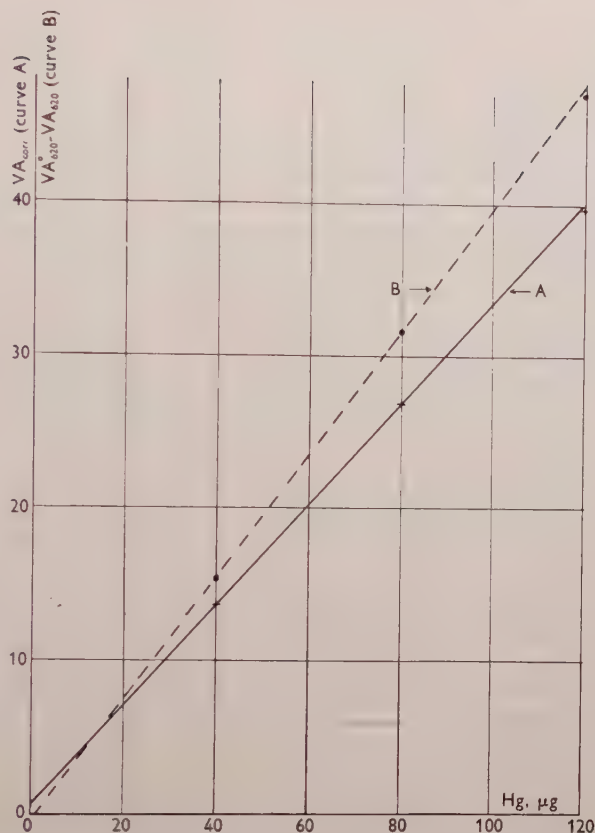


Fig. 5. Standard curves with chloroform as solvent. Calculated by method A: $k_A = 0.33$, by method B: $k_B = 0.40$.

given in per cent of the mercury added. The recovery was about 97 %. When no interfering substances were present the differences in the results calculated according to methods A and B were insignificant.

As unknown samples have not been analysed in this investigation the blank values were not obtained by digestion of sucrose but by the analysis of a similar quantity of the same material as in the corresponding samples, but without the addition of mercury. The blank values usually corresponded to 1–4 μg mercury. These could be due to the presence of mercury, copper or noble metals in the material and reagents, and to other circumstances in the analytical procedure. Thus the values are throughout higher when calculated by method B than by method A, presumably due to some oxidation or destruction of the dithizone. The blank values for liver and kidney are usually higher than for muscle.

Experiments with the addition of anions forming complexes with mercury revealed that considerable disturbances occurred only with relatively large additions (Table 4). The following substances were used in these experiments: NaCl, KBr, KI, NH_4SCN and $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5 \text{H}_2\text{O}$.

Table 3. Estimations with carbon tetrachloride as solvent.

Weighed		Hg added μg	Agitation time for sample, min.	Calculated by A				Calculated by B			
Material	g			Sample Hg μg	Blank Hg μg	Difference Hg μg	Recovery %	Sample Hg μg	Blank Hg μg	Difference Hg μg	Recovery %
Cow muscle	25	10	1	12.2	2.6	9.6	96	13.6	3.9	9.7	97
"	"	10	2	11.7	2.1	9.6	96	14.2	4.5	9.7	97
"	"	10	2	11.1	1.6	9.5	95	13.9	3.3	10.6	106
"	"	10	1	11.0	1.6	9.4	94	13.0	3.3	9.7	97
"	"	20	2	21.9	1.7	20.2	101	24.2	3.9	20.3	101.5
"	"	20	3	21.3	2.1	19.2	96	23.0	4.5	18.5	92.5
"	"	20	3	20.4	1.6	18.8	94	23.0	3.3	19.7	98.5
"	"	25	3	24.0	0.6	23.4	94	25.8	2.7	23.1	92
"	"	40	1	41.6	1.6	40.0	100	42.4	3.3	39.1	98
"	"	50	2	49.1	0.6	48.5	97	51.2	2.7	48.5	97
"	"	60	3	58.9	0.6	58.3	97	61.2	2.7	58.5	97.5
"	"	60	2	62.0	1.6	60.4	101	64.8	4.2	60.6	101
"	"	60	1	59.7	1.1	58.6	98	61.2	2.7	58.5	97.5
"	"	60	3	60.7	0.9	59.8	100	62.4	2.4	60.0	100
"	"	80	1	80.5	0.6	79.9	100	81.5	2.7	78.8	98.5
Swine liver	30	30	1	33.1	3.2	29.9	100	34.8	4.8	30.0	100
"	"	40	1	41.8	4.0	37.8	94.5	43.9	6.4	37.5	94
"	"	50	1	51.9	4.0	47.9	96	53.3	6.4	46.9	94
"	"	60	1	62.3	3.2	59.1	98.5	63.3	4.8	58.5	97.5
Swine kidney	40	40	1	47.5	8.3	39.2	98	49.1	9.1	40.0	100
"	"	50	1	55.7	8.3	47.4	95	57.3	9.1	48.2	96
Mean							97.2				97.7

Table 4. Effect of anions. Solvent carbon tetrachloride.

Weighed		Added		Agitation time for sample, min.	Calculated by A				Calculated by B				Notes
Material	g	Anion	mg	Hg μ g	Sample Hg μ g	Blank Hg μ g	Difference Hg μ g	Recovery %	Sample Hg μ g	Blank Hg μ g	Difference Hg μ g	Recovery %	
Cow muscle	25	Cl ⁻	1000	60	58.5	0.8	57.7	96	62.7	2.1	60.6	101	Br ⁻ added after combustion
"	"	"	2000	60	59.3	1.1	58.2	97	63.0	2.4	60.6	101	
"	"	"	5000	60	66.5	1.1	65.4	109	75.5	2.4	73.1	122	
"	"	Br ⁻	500	60	55.5	1.0	54.5	91	64.2	1.8	62.4	104	
"	"	"	500	60	58.5	1.3	57.2	95	73.9	3.9	70.0	117	I added after combustion
"	"	"	500	60	56.7	1.0	55.7	93	59.1	3.0	56.1	93.5	
"	"	I ⁻	500	60	57.8	1.0	56.8	95	58.5	3.0	55.5	92.5	
"	"	"	500	60	59.1	1.0	58.1	97	62.1	1.8	60.3	100.5	
"	"	"	500	60	58.8	1.3	57.5	96	60.6	3.9	56.7	94.5	SCN ⁻ added after combustion
"	"	"	500	60	57.5	1.0	56.5	94	57.9	3.0	54.9	91.5	
"	"	SCN ⁻	500	60	55.8	1.3	54.5	91	57.9	3.6	54.3	90.5	
"	"	"	500	60	58.6	3.3	55.3	92	59.4	6.4	53.0	88	
"	"	"	500	60	56.4	1.3	55.1	92	57.6	3.6	54.0	90	S ₂ O ₃ ²⁻ added af- ter combustion
"	"	"	500	60	59.6	3.3	56.3	94	59.4	6.4	53.0	88	
"	"	S ₂ O ₃ ²⁻	500	60	29.6	1.3	28.3	47	31.2	3.6	27.6	46	
"	"	"	50	60	58.8	1.3	57.5	96	62.1	3.6	58.5	97.5	
"	"	"	225	60	53.6	1.4	52.2	87	52.4	3.9	48.5	81	S ₂ O ₃ ²⁻ added af- ter combustion
"	"	"	50	60	55.9	1.3	54.6	91	57.0	3.6	53.4	89	

Table 5. Effect of metals. Solvent carbon tetrachloride.

Weighed		Added		Agitation time for sample, min.	Calculated by A				Calculated by B				Note
					Sample Hg μg	Blank Hg μg	Difference Hg μg	Recovery %	Sample Hg μg	Blank Hg μg	Difference Hg μg	Recovery %	
Cow muscle	25	Cr ^{VI}	50	2	61.7	1.6	60.1	100	62.4	3.3	59.1	98.5	(Dithizone solution orange-yellow)
"	"	Mn ^{VII}	50	1	60.7	1.7	59.0	98	62.7	3.9	58.8	98	
"	"	Fe ^{II}	50	2	60.4	1.6	58.8	98	63.9	4.2	59.7	99.5	
"	"	Co ^{II}	50	2	60.2	1.7	58.5	97.5	62.7	3.9	58.8	98	
"	"	Ni ^{II}	50	2	59.6	1.6	58.0	97	62.4	4.2	58.2	97	
"	"	Ag ^I	0.1	1									
"	"	Zn ^{II}	50	1	60.7	2.6	58.1	97	61.2	3.9	57.3	95.5	
"	"	Cd ^{II}	50	2	60.2	1.7	58.5	97.5	64.2	3.9	60.3	100.5	
"	"	Al ^{III}	50	1	59.9	2.6	57.3	95.5	60.9	3.9	57.0	95	
"	"	Ti ^{IV}	50	1	60.4	2.6	57.8	96	63.0	3.9	59.1	98.5	
"	"	Sn ^{II}	50	2	59.4	1.6	57.8	96	60.0	4.2	55.8	93	
"	"	Pb ^{II}	50	3	59.6	1.6	58.0	97	61.8	4.2	57.6	96	
"	"	As ^{III}	50	2	62.3	2.1	60.2	100	63.9	4.5	59.4	99	
"	"	Bi ^{III}	50	1	60.9	0.6	60.3	100.5	62.4	2.7	59.7	99.5	
"	"	Ca ^{II}	50	1	60.6	2.6	58.0	97	61.8	3.9	57.9	96.5	
Mean								97.6				97.5	

Table 6. Estimations with chloroform as solvent.

Weighed		Added		Agitation time for sample, min.	Calculated by A				Calculated by B				Notes
Material	g	Metal	mg		Hg μg	Sample Hg μg	Blank Hg μg	Difference Hg μg	Recovery %	Sample Hg μg	Blank Hg μg	Difference Hg μg	
Cow muscle	25	—	—	10	12.4	2.3	10.1	101	14.0	4.0	10.0	100	Absorption not constant
"	"	—	—	15	15.9	1.4	14.5	97	16.7	3.0	13.7	91	Absorption not constant
"	"	—	—	20	24.1	3.4	20.7	103.5	24.0	4.5	19.5	97.5	
"	"	—	—	40	41.1	2.3	38.8	97	43.5	4.0	39.5	99	
"	"	—	—	60	63.8	3.2	60.6	101	64.2	5.5	58.7	98	
"	"	—	—	100	106.9	3.2	103.7	104	103.5	5.5	98.0	98	
"	"	C ₂ H ₃ VI	50										
"	"	Ag ^I	0.1	60									
"	"	Cd ^{II}	50	75	76.8	2.6	74.2	99	76.7	5.0	71.7	96	Emulsion formed (Dithizone orange- yellow)
"	"	Al ^{III}	50	60	58.9	2.6	56.3	94	61.5	5.0	56.5	94	
"	"	Bi ^{III}	25	—	1.6	2.6	-1.0		2.5	5.0	-2.5		
"	"	"	10	75	77.0	2.6	74.4	99	78.2	5.0	73.2	98	
"	"	"	25	75	74.7	2.6	72.1	96	79.7	5.0	74.7	100	
Mean								99.2					97.2

Chloride had no effect up to 2 grams Cl^- , but at 5 g the results were too high. The high value from method B may be due to the formation of oxidising agents during combustion, probably active chlorine compounds, which could not be entirely destroyed by treatment with hydroxylamine. These may have caused destruction of dithizone through oxidation.

In the presence of *bromide* (500 mg Br^-) the results were possibly too low by method A but too high by method B. The addition of *iodide* (500 mg I^-) did not appear to cause any great complication. *Thiocyanate* (500 mg SCN^-) caused fairly low results, with a recovery of approx. 90%. *Thiosulfate* in small quantities (50 mg $\text{S}_2\text{O}_3^{--}$) did not appear to have any appreciable effect, but larger quantities (500 mg $\text{S}_2\text{O}_3^{--}$) resulted in a recovery less than 50%.

Experiments on the effect of extraneous metals are presented in Tables 5 and 6. The metals were added as nitrates or sulfates. *Silver* was extracted simultaneously with mercury and thus caused considerable disturbances. According to VAŠÁK and ŠEDIVÉČ (5) silver can, however, be masked by addition of potassium thiocyanate. The presence of metals more noble than silver, e.g. gold, platinum and palladium, would make analysis impossible, but no samples containing these metals were analysed. *Chromium* (50 mg) had no effect on determination with dithizone in carbon tetrachloride, but with dithizone in chloroform analysis was made impossible by the formation of an emulsion. Other metals tested except copper (Mn, Fe, Co, Ni, Zn, Cd, Al, Tl, Sn, Pb, As, Bi and Ca) did not appreciably affect the analysis in quantities up to 50 mg.

The effect of copper

Copper interferes relatively markedly in extraction but not to the same extent as the noble metals. As copper is commonly present in biological materials it was found necessary to further investigate its effect on the analysis.

VAŠÁK and ŠEDIVÉČ (5) found that the presence of copper resulted in too high mercury recoveries, that the error due to copper was directly proportional to the time of agitation and that the best results were obtained with as short a period of agitation as possible.

In a series of experiments 25 g of muscle were combusted after the addition of up to 10 mg of copper as nitrate. Three levels of mercury content were used: 0, 20 and 60 μg . The extraction was performed with carbon tetrachloride as solvent for the dithizone and the results were calculated after 1 and 2 minutes agitation according to methods A and C. The blank values for muscle alone were subtracted as previously. The results are shown in Figs. 6–8 as four curves, 1A, 1C, 2A and 2C. Of these 1A was nearest the actual mercury content. The results of these experiments lead to the following views on the effect of copper.

1) The absorbancies do not become constant even after several minutes agitation, as the versene does not prevent but only retards the extraction of copper. Even after a definite agitation time it is difficult to obtain reproducible values, as is apparent from the irregular course of the curves.

2) On calculating the results by methods B or C considerably higher results are obtained than by method A. This is due to the absorption maximum of copper dithizonate lying at 550 $\text{m}\mu$ and there being relatively low absorption at 500 $\text{m}\mu$. Therefore calculations by method A are not so greatly affected.

3) In the presence of copper in large quantities the extract turns first reddish and then violet-red.

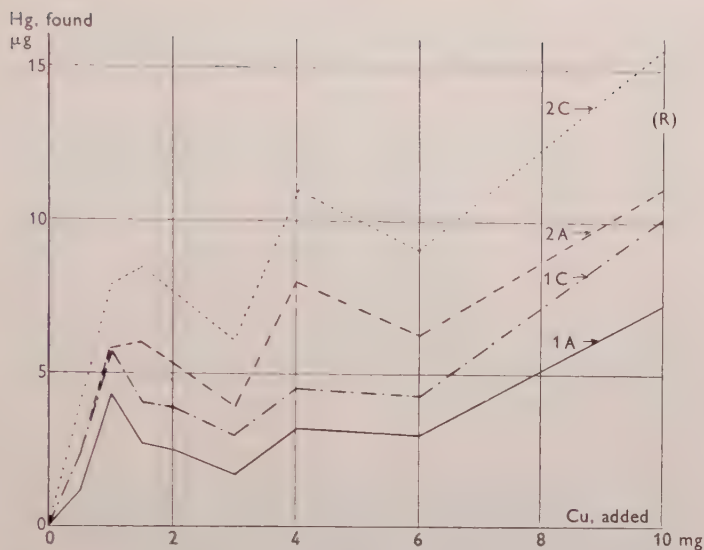


Fig. 6. Extraction in the presence of copper with carbon tetrachloride as solvent. No addition of mercury.

Key to Figs. 6-8:

1A and 1C: extraction 1 min., calculated by methods A and C respectively.

2A and 2C: extraction 2×1 min., calculated by methods A and C respectively.

(R): the extract had a reddish tinge.

R: the extract was reddish to violet-red.

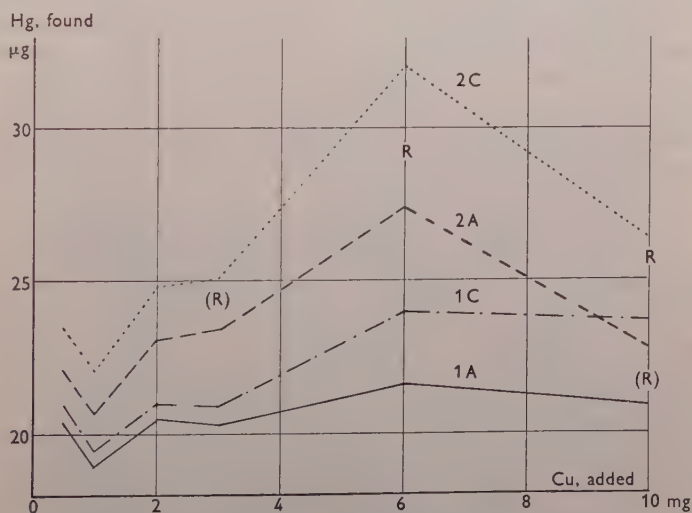


Fig. 7. Extraction in the presence of copper with carbon tetrachloride as solvent. 20 µg mercury added.

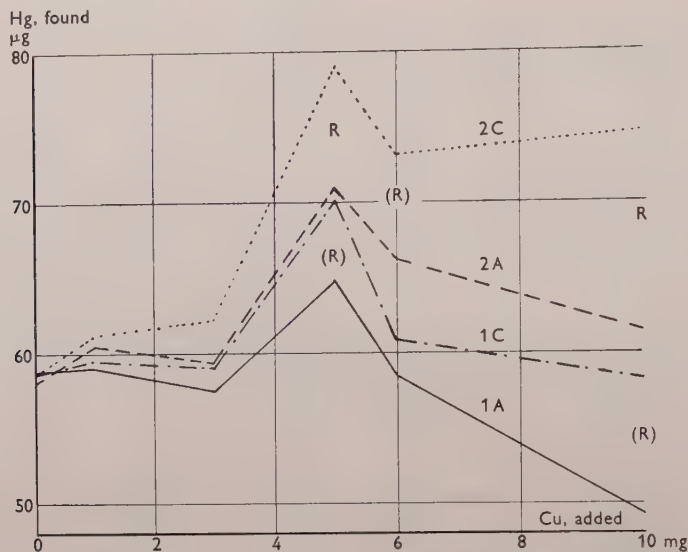


Fig. 8. Extraction in the presence of copper with carbon tetrachloride as solvent. 60 μg mercury added.

4) Up to 2 or 3 mg of copper (equivalent to 80 or 120 mg of copper per kg material when 25 g are weighed) could be present without making the results too unreliable. Copper in quantities of less than 0.25 mg hardly affect the analysis noticeably. With chloroform as solvent similar results were obtained.

Procedure in the presence of copper. In the presence of copper the same procedure as previously is used with the difference that the separatory funnel is agitated vigorously for only one minute. The results are calculated by method A. If the extract becomes reddish, then the analysis should be less reliable, and if it becomes violet-red then the analysis should not be continued.

When these investigations were finished H. FRIEDEBERG (10), who also used versene as masking agent, reported that he was able to determine mercury in presence of large amounts of copper by a decomposition-reextraction method.

Identification of mercury

The quantitative estimation of mercury does not provide evidence for the presence of mercury. Noble metals and copper can react with dithizone even in the presence of versene, and other metals can be present in greater quantities than the versene can mask. It is therefore desirable to confirm the presence of mercury qualitatively.

A. STOCK and co-workers (11-13) have elaborated a method for the micro-estimation of mercury in amounts less than 0.1 μg in organic material after the mercury has been obtained in metallic form. As under the present circumstances it was not necessary to demonstrate much less than 10 μg , the method could be simplified in some respects.

The qualitative test was applied to the dithizone solution, used in the quantitative determination, after the dithizone had been destroyed in a way similar to that used

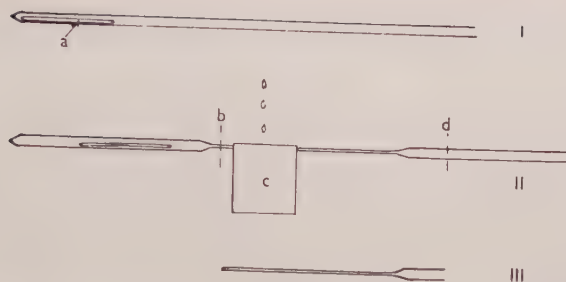


Fig. 9. Capillary tube for distillation of mercury (A. Stock and co-workers (11-13)).



Fig. 10. Arrangement for distillation of mercury.

by ALLPORT and SKRIMSHIRE (14). As described below, 10 to 100 μg mercury can be detected in dithizone solution with good results. No measurements were made on the mercury drops.

Procedure. After quantitative estimation the dithizone solution is evaporated in vacuo. When only a few ml remain the solution is transferred to a thick-walled pyrex test tube (length 150 mm, outer diam. 20 mm) with a ground opening (19/38). A couple of small boiling stones are added and evaporation is continued to dryness in vacuo. The residue is added with 1 ml of acid mixture and combusted under a Liebig condensor (length 300 mm) with a microburner for 30–45 minutes. After cooling the contents are transferred quantitatively, by rinsing with a little water, into a 30 ml beaker. 7 ml water is placed in the combustion tube and, with the condenser in place but with the water turned off, the apparatus is boiled again until the condensing fumes have risen to about the middle of the condenser. After cooling this fluid is also poured into the small beaker and the volume adjusted to about 25 ml.

An electrolytic copper wire (diam. 0.8–1 mm) is rinsed in nitric acid (1 + 2) and then in water. The wire is immersed until it nearly reaches the bottom of the beaker (immersed part = about 25 mm) and connected to the negative pole of an accumulator (4 volts). The beaker is covered with two halves of a watch-glass and electrolysed with a platinum coil as anode at 0.007 amp (1 amp/dm²) overnight. Then the cathode is removed, washed with water, alcohol and ether and then left for $\frac{1}{2}$ to 1 hour to dry in a desiccator.

A tube of ordinary soda glass (int. diam. 1.8 mm, ext. diam. 3.6 mm, length 150 mm), which has been treated in dichromate-sulfuric acid and then rinsed with water, alcohol and ether, is closed by melting in one end. The tube is then left to cool and dry in a desiccator. The copper wire is clipped off just above the place the fluid surface reached, and the part with the mercury deposit is placed at the bottom of the tube (Fig. 9, Ia). The tube is heated 6–7 cm from the closed end so that the copper wire is not heated, and drawn out in a capillary 6–7 cm long (Fig. 9, II). The tube is placed on a slightly V-shaped rack made of a few iron wires (Fig. 10). Over the capillary near the copper wire a piece of filter paper is folded and kept continually moist by dropping water (Fig. 9, IIc). The tube end with the copper wire is heated first carefully and is then fused from the closed end towards the capillary. When the flame reaches the capillary it is reduced and the capillary is melted off immediately beside the filter paper (Fig. 9, IIb). The tube is then cut off at the other end (Fig. 9, IIId), so that a capillary, containing the condensed mercury, with one end open and wide remains (Fig. 9, III). A few drops of alcohol are placed in the open end with a capillary pipette. The capillary tube is enclosed in cotton-wool, centrifuged at 3000 rev/min for 10–15 minutes and is then inspected with a magnifying glass or a preparation microscope. Any mercury present will have collected into one or a few drops. A control sample is examined from the blank dithizone extract to check the possibility of introduction of mercury into the sample from the utensils.

Summary

The method of determination of mercury with dithizone and with versene as masking agent proposed by V. VAŠÁK and V. ŠEDIVÉČ (5) was tested and modified for the determination of mercury in biological materials. 10–100 μg mercury in 25 g of organ from animals could be determined with a mean recovery of 97 %.

The effect of a number of anions and cations on the analysis was investigated. Serious interference occurred only in the presence of silver and copper. The effect of copper was investigated more extensively.

A procedure for the identification of mercury in dithizone extract based on a method elaborated by A. STOCK and co-workers (11-13) was tried out.

The authors wish to thank Mrs. C. ANSGÅRD and Mrs. A. G. KUYLSER who have taken an important part in the experimental work.

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Considerations of the kinetics of respiration¹

Respiration a reaction via carrier chains

By BARBRO GRABE

With 3 figures in the text

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Summary

The kinetics of respiration via carriers are treated. The methods and results can be applied to other reactions of the same type. The calculations are made on the assumption that the carrier chain is branched to different substrates.

¹ A paper on the same subject but without mathematical deductions is to be published in *Experimental Cell Research*.

The steps in the chains are characterized by constants, R_k , which indicate the maximum velocity that can pass this step.

In the general case, with an arbitrary number of carriers in the common chain as well as in the branches, equations are given which connect the O_2 pressure and the R_k with the rates of reaction in the common chain and in the branches.

If there are no carriers in the branches, the ratios between the reaction rates in the branches depend only on the constants in the branches. If there is at least one carrier in any branch, the ratios between the rates in the branches depend in addition on the respiration velocity, so that a change only in the common chain alters the ratios between the rates in the branches. The values of the R_k in the branches may be such that the rate in one of the branches dominates at low respiration while that in another dominates at a faster respiration.

The special case of cytochrome oxidase and one carrier in the common chain and 2 branches with one carrier in each is treated particularly.

If the $R_k^{(m)}$ constants for $k > n$ are very much larger than $R_n^{(m)}$, the respiration goes on as if the branch ended with the n th carrier, and a further increase of the carriers and substrate corresponding to these large $R_k^{(m)}$ has no influence on the rates as in the case of unbranched chains [1].

The difference in respiration at points at different distances from the periphery of the cell is discussed. The significance of the different R_k constants, if e.g. the diffusion constant or the dimensions of the cell are changed, is indicated.

The back reactions are considered in some simple cases with 2 branches. Even if there are no carriers in the branches the ratio between the rates of oxidation of the substrates depends on the respiration velocity. If the velocity in the common chain is below a certain value one of the substrates is reduced.

To show the significance of the ratio between the rates in the branches, a case is treated where there are 2 branches and the substrates, A' and A'' , are intermediates in the metabolism of a substance. Sometimes even small changes in the R_k constants in the branches and in the common chain may cause large changes in the concentration of A'' , the second of the intermediates, at the steady state. If A'' besides being oxidized, can react according to $A'' \rightarrow F$, the rate of this reaction may be greatly changed. If $A'' \rightarrow F$ is a step in the formation or stimulation of certain carriers, $A'' \rightarrow F$ may be a regulation reaction.

I. Introduction

The kinetics of respiration reactions are treated. The methods and results can probably be applied to other reactions of the same type, e.g. phosphate transfer. The characteristics of the type may be evident from the following description of respiration. When a substance, called the substrate, is to be oxidized by free oxygen, a number of enzymes, called carriers, are necessary. An enzyme, cytochrome oxidase (denoted Warburg enzyme in [1]), is oxidized by free oxygen, and the oxidized enzyme is reduced by carrier No. 1. Then cytochrome oxidase is reoxidized by free oxygen. Carrier No. 1 in the oxidized state is reduced by carrier No. 2 and so on.¹ In this manner the oxidation is transferred along the chain of carriers to the substrate

¹ Really cytochrome oxidase is a carrier too. For practical reasons it is not included in the chain of carriers, when they are numbered, but it is always included in the term "the carriers" and the "carrier chain".

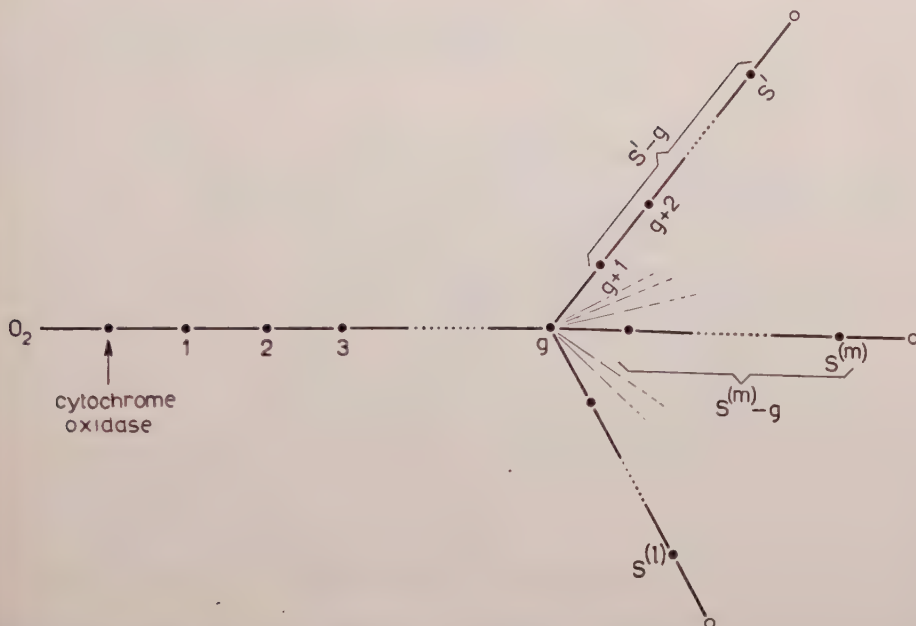


Fig. 1. A figurative chain. The points represent the carriers and the circles the substrates. The notation for the number of carriers and branches is given in Chapter II. $s' - g$, $s^{(m)} - g$ and $s^{(l)} - g$ are the numbers of carriers in the branches.

at the end of the chain.¹ Different chains of carriers are necessary for the oxidation of different substrates. The calculations of this article have been carried out under the assumption that cytochrome oxidase and the nearest g carriers are common to the oxidations of some substrates, but that the chain is then branched to the different substrates (Fig. 1). The unbranched respiration chain of carriers is treated by KLEIN-RUNNSTRÖM [1], and this paper forms a sequel.

The oxidized state of a carrier can also be reduced by the preceding carrier in the chain. This is called a back reaction. In general the rates of these reactions are small in comparison to those of the direct reactions. In the main part of this paper the back reactions are not considered. In [1] the effect of neglecting them is discussed quantitatively. Similar calculations can be applied to branched chains too.

Diffusion is not considered in the equations which relate O_2 pressure and respiration velocity. It is, however, considered, when the conditions for respiration at different distances from the periphery of the cell are discussed.

¹ The carriers are numbered from the O_2 side of the chain. This is useful as the O_2 side of the chain is common to all the oxidations considered. It should be stressed that this notation has nothing to do with the fact that hydrogen or electrons are transferred along the chain. The only assumption made is that a carrier k is oxidized by a carrier, numbered $k - 1$, and therefore called preceding (in the figurative chain), and reduced by a carrier, numbered $k + 1$, and therefore called subsequent.

II. Notation

The following notation is used, and is chosen in agreement with the notation in [1].

l	The number of branches
g	the number of carriers in the common chain, excluding cytochrome oxidase
$s^{(m)} - g$	the number of carriers in the m th branch (Fig. 1) If all $s^{(m)}$ are equal, $s' = s'' = \dots = s$
p	partial pressure of O_2 in the atmosphere
α	absorption coefficient of O_2
αp	concentration of O_2 in solution
a_0	total concentration of cytochrome oxidase
$a_0 x_0$	concentration of reduced cytochrome oxidase
$a_0(1 - x_0)$	concentration of oxidized cytochrome oxidase
$a_1 x_1, \dots, a_g x_g$	concentration of respective carriers in reduced state
$a_1(1 - x_1), \dots, a_g(1 - x_g)$	concentration of respective carriers in oxidized state

The quantities belonging to the m th branch have a superscript.

$a_{g+1}^{(m)} x_{g+1}^{(m)}, \dots, a_{s^{(m)}}^{(m)} x_{s^{(m)}}^{(m)}$	concentration of respective carriers in the m th branch in reduced state
$a_{g+1}^{(m)}(1 - x_{g+1}^{(m)}), \dots, a_{s^{(m)}}^{(m)}(1 - x_{s^{(m)}}^{(m)})$	concentration of respective carriers in the m th branch in oxidized state
$a_{s^{(m)+1}}^{(m)}$	concentration of the substrate in the m th branch in reduced state
λ_0	reaction velocity constant for the oxidation of cytochrome oxidase by O_2
λ_1	reaction velocity constant for the oxidation of carrier No. 1 by cytochrome oxidase
$\lambda_{g+k}^{(m)}$	reaction velocity constant for the oxidation of the k th carrier in the m th branch by the $(k-1)$ th carrier of the branch
$\lambda_{s^{(m)+1}}^{(m)}$	reaction velocity constant for the oxidation of the substrate in the m th branch by the last ($s^{(m)}$ th) carrier
R	total respiration velocity, i.e. the rate in the common chain
$q^{(m)} = \theta^{(m)} R$	the reaction velocity in the m th branch.

Further

$$R_0 = \lambda_0 \alpha a_0, R_1 = \lambda_1 a_0 a_1, \dots, R_g = \lambda_g a_{g-1} a_g, \dots, R_{g+k}^{(m)} = \lambda_{g+k}^{(m)} a_{g+k-1}^{(m)} a_{g+k}^{(m)}, \dots$$

$$R_{s^{(m)+1}}^{(m)} = \lambda_{s^{(m)+1}}^{(m)} a_{s^{(m)}}^{(m)} a_{s^{(m)+1}}^{(m)} \dots \quad (1)$$

and

$$M^{(m)} = \frac{R_s^{(m)}}{R_{s+1}^{(m)}} = \frac{R'_s}{R'_{s+1}} \quad (2)$$

R_k signifies the maximum velocity which is possible at this step of the chain (cf. p. 33).

In the main part of the paper steady states are considered and then all the R_k are constants during the reaction. In chapter IX, however, the concentration of one of the substrates changes during the reaction (R'_{s+1} varies), but all the other R_k are constants.

III. General case.

An arbitrary number of carriers in the common chain as well as in the branches, an arbitrary number of branches

R is the total respiration velocity and $\varrho^{(m)} = \theta^{(m)} R$ is the reaction velocity in the m th branch. If the back reactions are neglected, the following equations are valid in the steady state. For $m = 1$

$$\begin{aligned} \varrho' = \theta' R = \theta' R_0 p x_0 = \theta' R_1 (1 - x_0) x_1 = \cdots = \theta' R_g (1 - x_{g-1}) x_g = R'_{g+1} (1 - x_g) x'_{g+1} \\ = R'_{g+2} (1 - x'_{g+1}) x'_{g+2} = \cdots = R'_{s+1} (1 - x'_{s+1}). \end{aligned} \quad (3)$$

Analogous equations hold for the other branches. Further

$$1 - x_g = \frac{\theta' R}{R'_{g+1} x'_{g+1}} = \frac{\theta'' R}{R'_{g+1} x'_{g+1}} = \cdots = \frac{\theta^{(l)} R}{R'_{g+1} x^{(l)}_{g+1}}, \quad (4)$$

because $1 - x_g$ for the last common carrier g , obtained from (3), must be the same whichever branch is used for the calculation, and

$$\sum_{m=1}^l \theta^{(m)} = 1, \quad (5)$$

because

$$\sum \varrho^{(m)} = R.$$

From the definition of x_k it is seen that $0 \leq x_k \leq 1$. Then R_k is the maximum velocity that is possible at the step " k " of the chain (see (3)). $R \rightarrow R_m$, when all the R_k for $k \neq m$ are $\gg R_m$, so that (i) $x_{m-1} \rightarrow 0$, i.e. the carrier $m-1$ is approximately kept in the oxidized state by the preceding chain, and (ii) $x_m \rightarrow 1$, i.e. the carrier m is approximately kept in the reduced state by the subsequent chain. Thus all the R_k except R_m are so large that only R_m is the limiting step. This same is also valid for the rates in the branches.

The problem is to find out how R and $\varrho^{(m)}$ depend on p and on the constants $R_0, R_1, \dots$.

It is possible to express

$$x_k^{(m)} = \frac{P_k^{(m)}(\theta^{(m)}, R)}{P_{k+1}^{(m)}(\theta^{(m)}, R)}, \quad (6)$$

where $P_k^{(m)}$ are polynomials in R (cf. [1]). In the general case $\theta^{(m)}$ are very complicated functions of R .

From (6) and (3) we obtain

$$P_{s+1}^{(m)} = 1; \quad P_s^{(m)} = 1 - \frac{\theta^{(m)} R}{R_{s+1}^{(m)}}; \quad P_{k-1}^{(m)} = P_k^{(m)} - \frac{\theta^{(m)} R P_{k+1}^{(m)}}{R_k^{(m)}} \quad \text{for } k > g \quad (7)$$

and

$$P_{k-1}^{(m)} = P_k^{(m)} - \frac{R P_{k+1}^{(m)}}{R_k} \quad \text{for } k \leq g. \quad (7)$$

It is also possible to obtain the $P_k^{(m)}$ from the corresponding P_k in [1] by substituting all the R_k for $k > g$ in the latter by $R_k^{(m)}/\theta^{(m)}$.

From (3) there is further obtained

$$p = \frac{R}{R_0} \frac{P_1^{(m)}}{P_0^{(m)}}. \quad (8)$$

It is also useful to consider

$$\frac{R(1-p)}{p} = R_0 \frac{P^{(m)}}{P_1^{(m)}}, \quad (9)$$

where

$$P^{(m)} = P_0^{(m)} - \frac{R}{R_0} P_1^{(m)}. \quad (10)$$

An arbitrary branch can be used for the calculations.

It remains to determine the relations between $\theta^{(m)}$ and R , by substituting (6) and (7) in (4) and (5). If there is more than one carrier in each branch, the equations become very complicated. Therefore the calculations are limited to the cases with no carriers or one in each branch.

IV. $\theta^{(m)}$ as function of the velocity.

An arbitrary number of carriers in the common chain, an arbitrary number of branches, no carriers or one in each branch

i. No carriers in the branches. ($s = g$)

Here the expression $1 - x_g$ for the different branches can be taken directly from (3).

$$1 - x_g = \frac{\theta' R}{R_{s+1}'} = \dots = \frac{\theta^{(l)} R}{R_{s+1}^{(l)}}$$

and this together with (5) gives

$$\theta^{(m)} = \frac{R_{s+1}^{(m)}}{\sum_{k=1}^l R_{s+1}^{(k)}}. \quad (11)$$

Thus the ratios between the reaction rates in the different branches ($\varrho':\varrho'':\dots$) depend only on the R_k in the branches and not on p or on the R_k in the common chain.

ii. One carrier in each of the branches. ($s - g = 1$)

From (4) and (6) it appears, that

$$\frac{\theta' P'_{s+1}}{R'_s P'_s} = \dots = \frac{\theta^{(l)} P^{(l)}_{s+1}}{R^{(l)}_s P^{(l)}_s}, \quad (12)$$

where according to (7)

$$P^{(m)}_{s+1} = 1 \quad \text{and} \quad P^{(m)}_s = 1 - \frac{R \theta^{(m)}}{R^{(m)}_{s+1}}.$$

Expressing all the $\theta^{(m)}$ in e.g. θ' we obtain

$$\theta^{(m)} = \frac{\theta' R^{(m)}_s}{R \theta' \left[\frac{R^{(m)}_s}{R^{(m)}_{s+1}} - \frac{R'_s}{R'_{s+1}} \right] + R'_s}.$$

Using the notation

$$\frac{R^{(m)}_s}{R^{(m)}_{s+1}} - \frac{R_s}{R'_{s+1}} = M^{(m)} \quad (13)$$

and (5) we obtain

$$\frac{1}{\theta'} = 1 + \sum_{m=2}^l \frac{R^{(m)}_s}{\theta' M^{(m)} + R'_s}. \quad (14)$$

Then in this case the ratios between the reaction rates in the branches depend not only on the R_k in the branches but also on the respiration velocity, i.e. they also depend on p and on the R_k in the common chain. (To get relatively simple expressions, the fractional part θ' of R that passes through the branch is given as a function of the velocity in the branch, θ' , instead of as a function of R .) Obviously it is possible to change the ratios between the rates in the branches ($\theta':\theta'':\dots$) by changing the respiration velocity by changes only in the O_2 pressure and in the common chain. This is true also, when there is more than one carrier in each branch, but in this case the relationships are much more complicated.

If there is more than one substrate in the same branch, i.e. all their carriers are common, the ratios between their rates are independent of the velocity. Their oxidation rates can, however, be changed in proportion to the oxidation rates of other substrates. Then groups of reaction rates can be shifted towards the rates of other groups by changing the O_2 pressure or the common chain only.

For the special case of 2 branches and one carrier in each, (14) gives

$$\theta' = \frac{M'' \theta' + R'_s}{M'' \theta' + R'_s + R'_s}; \quad (15 \text{ a})$$

$$\frac{\theta'}{\theta''} = \frac{\theta'}{1 - \theta'} = \frac{M'' \theta' + R'_s}{R'_s}. \quad (15 \text{ b})$$

If there are no carriers in one of the branches, e.g. the m th branch, (14) and (15) are still valid. In this case $R^{(m)}_{s+1}$ is infinite and $R^{(m)}_s$ refers to the oxidation of the substrate by the last carrier (No. $s - 1$).

To find how θ' varies with ϱ' , the function

$$\frac{d\left(\frac{1}{\theta'}\right)}{d\varrho'} = \sum_{m=2}^l \frac{-R_s^{(m)} M^{(m)}}{(\varrho' M^{(m)} + R_s)^2} \quad (16)$$

is formed.

If there are only 2 branches, θ' obviously increases monotonously, is constant or decreases monotonously, when ϱ' increases. What happens depends on the value of M'' , if $M'' > 0$, $M'' = 0$ or $M'' < 0$. The terms R'_s/R'_{s+1} and R''_s/R''_{s+1} in M'' signify the ratios between the capacities for transmission of the two subsequent steps of oxidation. Obviously it is the difference between these ratios which is significant. When the velocity is very small, R'_s and R'_s determine the distribution between the branches (see (15b)), but when the velocity increases the second step, i.e. R'_{s+1} and R''_{s+1} have more influence. The magnitude of the influence depends on the ratios between the two oxidation steps.

If there are more than 2 branches, let one dash at first denote the branch in which R_s/R_{s+1} is smaller than in any other branch. Then θ' increases monotonously if the reaction rate increases. If, on the other hand, one dash denotes the branch in which R_s/R_{s+1} is larger than in any other branch, then θ' decreases when ϱ' increases. For the other branches $1/\theta^{(m)}$ is increasing in some intervals and decreasing in others.

If (14) and $R = \varrho'/\theta'$ are substituted in the $P^{(m)}$ functions (7) for $m = 1$, these are obtained as functions of ϱ' only. If these functions are substituted in (8) and (9), the dependence of ϱ' on p and on the R_k constants is obtained. Unfortunately the expressions become very complicated, if there are several carriers in the common chain. Therefore only a special case is calculated particularly.

V. The dependence of ϱ' on the O_2 pressure and on the R_k constants.

Cytochrome oxidase and one carrier in the common chain, 2 branches and one carrier in each branch. ($g = 1$; $s = g + 1 = 2$)

i. General deduction

From (7) the following equations are obtained.

$$P'_3 = P'_{s+1} = 1$$

$$P'_2 = P'_s = 1 - \frac{\varrho'}{R'_3}$$

$$P'_1 = P'_{s-1} = 1 - \left(\frac{1}{R'_3} + \frac{1}{R'_2} \right) \varrho' \quad (17)$$

$$P'_0 = P'_{s-2} = 1 - \left(\frac{1}{R'_3} + \frac{1}{R'_2} + \frac{1}{R'_1 \theta'} \right) \varrho' + \frac{\varrho'^2}{\theta' R'_1 R'_3} \quad (18)$$

$$P' = P'_{s-3} = 1 - \left(\frac{1}{R'_3} + \frac{1}{R'_2} + \frac{1}{R_1 \theta'} + \frac{1}{R_0 \theta'} \right) \varrho' + \\ + \left(\frac{1}{R_1 R'_3} + \frac{1}{R_0 R'_3} + \frac{1}{R_0 R'_2} \right) \frac{\varrho'^2}{\theta'} \quad (19)$$

and from (8) and (9)

$$p = \frac{\varrho' P'_1}{R_0 \theta' P'_0} \quad (20)$$

$$\frac{1-p}{p} = R_0 \frac{\theta' P'}{\varrho' P'_1} \quad (21)$$

By substituting (15 a) in (18) and (19) the following equations are obtained.

$$\theta' P'_0 = \frac{1}{M'' \varrho' + R'_2 + R'_2} \cdot \\ \cdot \left[M'' \varrho' + R'_2 - \varrho' \left(\frac{M'' \varrho' + R'_2}{R'_3} + \frac{M'' \varrho' + R'_2}{R'_2} + \frac{M'' \varrho' + R'_2 + R'_2}{R_1} \right) + \right. \\ \left. + \varrho'^2 \frac{(M'' \varrho' + R'_2 + R'_2)}{R_1 R'_3} \right];$$

$$\theta' P'_0 = \frac{1}{M'' \varrho' + R'_2 + R'_2} \left[\frac{\varrho'^3 M''}{R_1 R'_3} + \varrho'^2 \left\{ \frac{R'_2 + R'_2}{R_1 R'_3} - M'' \left(\frac{1}{R_1} + \frac{1}{R'_2} + \frac{1}{R'_3} \right) \right\} + \right. \\ \left. + \varrho' \left\{ M'' - \frac{R'_2 + R'_2}{R_1} - R'_2 \left(\frac{1}{R'_2} + \frac{1}{R'_3} \right) \right\} + R'_2 \right]; \quad (22)$$

$$\theta' P' = \frac{1}{M'' \varrho' + R'_2 + R'_2} \left[M'' \varrho' + R'_2 - \varrho' \left\{ (M'' \varrho' + R'_2) \left(\frac{1}{R'_2} + \frac{1}{R'_3} \right) + \right. \right. \\ \left. \left. + (M'' \varrho' - R'_2 + R'_2) \left(\frac{1}{R_0} + \frac{1}{R_1} \right) \right\} + \varrho'^2 (M'' \varrho' + R'_2 + R'_2) \left(\frac{1}{R_1 R'_3} + \frac{1}{R_0 R'_3} + \frac{1}{R_0 R'_2} \right) \right];$$

$$\theta' P' = \frac{1}{M'' \varrho' + R'_2 + R'_2} \left[\varrho'^3 M'' A + \varrho'^2 \left\{ A (R'_2 + R'_2) - M'' \left(\frac{1}{R_0} + \frac{1}{R_1} + \frac{1}{R'_2} + \frac{1}{R'_3} \right) \right\} + \right. \\ \left. + \varrho' \left\{ M'' - R'_2 \left(\frac{1}{R'_2} + \frac{1}{R'_3} \right) - (R'_2 + R'_2) \left(\frac{1}{R_0} + \frac{1}{R_1} \right) \right\} + R'_2 \right], \quad (23)$$

where

$$A = \frac{1}{R_1 R'_3} + \frac{1}{R_0 R'_3} + \frac{1}{R_0 R'_2} \quad (24)$$

The roots of $\theta' P'_0 = 0$ are denoted by $r'_0, r'_0/\mu$ and r'_0/ε , where r'_0 is the smallest positive root. The product of the roots is $r'^3_0/\mu\varepsilon = -R_1 R'_2 R'_3/M''$. The roots of $\theta' P' = 0$ are denoted in the same manner by $r', r'/\kappa$ and r'/ν , where r' is the smallest positive root. The product of the roots is $r'^3/\kappa\nu = -R'_2/A M''$. The root of $P'_1 = 0$ (17) is

$$r'_1 = \frac{1}{1/R'_2 + 1/R'_3} = \frac{R'_2 R'_3}{R'_2 + R'_3}. \quad (25)$$

From (20) and (21) it follows that the root r' is the rate in the first branch, when $p = 1$, and that the root r'_0 is the limit of ϱ' , when $p \rightarrow \infty$.

Introducing these values (20) and (21) give

$$p = \frac{\varrho' \left(1 - \frac{\varrho'}{r'_1}\right) (M'' \varrho' + R'_2 + R'_2')}{R_0 R'_2 \left(1 - \frac{\varrho'}{r'_0}\right) \left(1 - \frac{\varrho' \mu}{r'_0}\right) \left(1 - \frac{\varrho' \varepsilon}{r'_0}\right)} \quad (26)$$

and

$$\frac{1-p}{p} = \frac{R_0 R'_2 \left(1 - \frac{\varrho'}{r'}\right) \left(1 - \frac{\varrho' \kappa}{r'}\right) \left(1 - \frac{\varrho' \nu}{r'}\right)}{\varrho' \left(1 - \frac{\varrho'}{r'_1}\right) (M'' \varrho' + R'_2 + R'_2')}. \quad (27)$$

(26) shows how ϱ' depends on p and on the R_k constants. Since we also have (14), the problem is solved in principle. Since the equations are very complicated, we shall discuss some special cases below.

ii. Special cases

In the special case where R'_2, R'_3, R'_2' and R'_3' are moderate and R_1 is very large it follows from (22) and (25) that

$$r'_0 \rightarrow \frac{R'_2 R'_3}{R'_2 + R'_3} = r'_1; \quad (28 \text{ a}) \quad \frac{r'_0}{\mu} \rightarrow -\frac{R'_2}{M''}; \quad (28 \text{ b}) \quad \varepsilon \rightarrow 0. \quad (28 \text{ c})$$

If R_0 , too, is very large, it follows from (23) that also

$$r' \rightarrow \frac{R'_2 R'_3}{R'_2 + R'_3} = r'_1; \quad (29 \text{ a}) \quad \frac{r'}{\kappa} = -\frac{R'_2}{M''}; \quad (29 \text{ b}) \quad \nu \rightarrow 0. \quad (29 \text{ c})$$

In these cases it is useful to express p as

$$p = \frac{\varrho' (M'' \varrho' + R'_2 + R'_2')}{R_0 R'_2} \left[\frac{1 - B \left(1 - \frac{\varepsilon \mu \varrho'}{r'_0}\right)}{\left(1 - \frac{\varrho' \mu}{r'_0}\right) \left(1 - \frac{\varrho' \varepsilon}{r'_0}\right)} + \frac{B}{1 - \frac{\varrho'}{r'_0}} \right], \quad (30)$$

where

$$B = \frac{1 - r'_0/r'_1}{1 + \varepsilon \mu - \mu - \varepsilon}.$$

It can be proved (cf. [1]) that $r' < r'_0 < r'_1$. Then it is always possible for a given q' to choose R_1 large enough to make the terms containing B negligible in comparison to the other terms. On the other hand, with R_1 fixed, it is always possible to choose q' so close to r'_0 , that $p \rightarrow \infty$.

VI. Discussion of the relations between the respiration velocity, the O_2 pressure and the R_k constants

At branched respiration chains, as at unbranched chains [1], the respiration rate is approximately $R = R_0 p$, when p is very small. When $p \rightarrow \infty$, R approaches the smallest positive root of $P_0^{(m)} = 0$ asymptotically (see (7), (8) and Fig. 2). As this root is independent of R_0 , respiration at very large values of p is independent of R_0 , and only dependent on the other R_k .

If the velocity q' in a branch is plotted against p , a curve of the type of Fig. 2 is obtained. When p is very small, q' is approximately equal to $\theta'_0 p R_0$, where θ'_0 is the value of θ' , when $R = 0$.

In [1] the case is discussed, where all the R_k , for which $k > n$, in an unbranched chain are very much larger than R_n . Then respiration occurs as if the chain ended with the n th carrier. It is limited only by R_n and the preceding part of the chain, and a further increase of these large R_k at the end of the chain, e.g. an increase of the concentration of the substrate, has no influence on the respiration. A comparison may be made with a reaction with a single enzyme, where the reaction constant and the concentration of the enzyme limit the reaction velocity, so that it cannot exceed a certain value, however large the concentration of the substrate might be. The difference between the single enzyme reaction and a carrier reaction is that in the latter case the limit in R_n can be at an early step in the chain. Not even an increase in the last carriers can increase the rate above a certain value. The same is valid for branched chains. If all the $R_k^{(m)}$ for which $k > n$ are $\gg R_n^{(m)}$, then a further increase of these large $R_k^{(m)}$ has no influence on the reaction rate in the branch, provided that at least one R_k in each branch is moderate, so that the distribution between the branches is determined.

It follows from the preceding paragraph that the formulae deduced, when there is only one carrier and a substrate in each branch, are also valid if there are more carriers, provided that only the two carriers in the beginning of each branch limit the rate, and all the following $R_k^{(m)}$ are very large.

In Fig. 3 the rate in one branch, q' , is schematically plotted against αp (the O_2 pressure in solution) for the special case which is treated in the preceding chapter (2 branches, $g = 1$ and $s = 2$). Curve 1 is valid, when R_0/α and R_1 are very large in comparison to the R_k in the two branches. Curve 2 is valid, when R_0/α is moderate and R_1 is very large and curve 3 is valid, when R_0/α is very large and R_1 is moderate. The limit, r'_1 , of curve 1 depends on R'_2 and R'_3 only (see (25)). The limit of q' of curve 3 depends on R_1 , R'_2 , R'_3 , R''_2 and R'_3 . It should be pointed out that each value of q' in Fig. 3 corresponds to a certain value of θ'/θ'' , since the R_k in both branches are unchanged. (See (15b).)

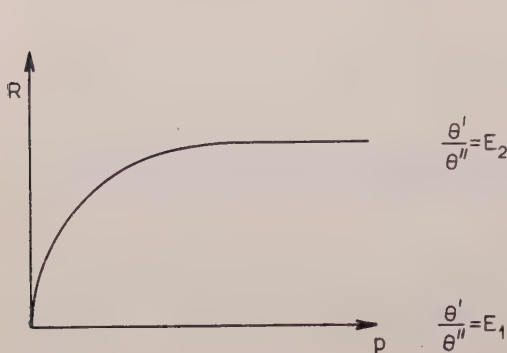


Fig. 2.

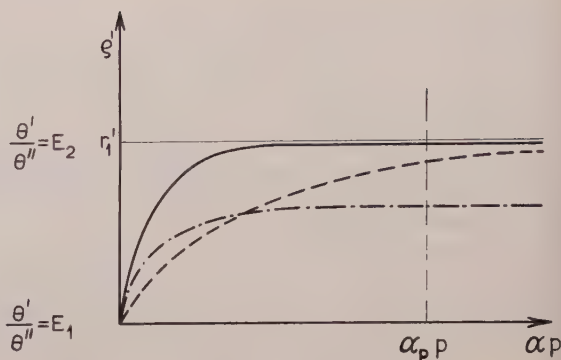


Fig. 3.

Fig. 2. A schematic curve showing the variation of respiration velocity R with the O_2 pressure p .

Fig. 3. Schematic curves showing the dependence of the oxidation rate in a branch q' on the O_2 pressure in solution, αp , with different values for the constants in the common chain (see p. 39). αp is defined on p. 40. E_1 and E_2 are the values of θ'/θ'' , when $q' = 0$ ($R = 0$) and $q' \rightarrow r_1'$ (see p. 41).

- R_0/α and R_1 very large. (Curve 1.)
- - - - - R_0/α moderate and R_1 very large. (Curve 2.)
- · - · - · - R_0/α very large and R_1 moderate. (Curve 3.)

On the other hand, if we inhibit in the branch in Fig. 3 when R_0/α and R_1 are very large, we obtain a curve of the same type as curve 3, but in this case the q' values do not correspond to the same values of θ'/θ'' as in Fig. 3. The slope of the curve at $p = 0$ is influenced by the changed value of θ' .

Figs. 2 and 3 can be considered as cell diagrams, showing the rates of oxidation at points at different distances from the periphery of the cell. Some facts can be noticed which are of importance for the regulation of rates. They depend on the fact that R and $q^{(m)}$ are functions of p and of the R_k and that the same is valid for the partial derivatives of R and $q^{(m)}$ with respect to p and the R_k . αp is assumed to be the only factor which is unequal in different parts of the cell. This very rough approximation is made to make the properties of the carrier chains evident. It does not invalidate the discussion, because the intent is only to give a schematic survey of the relationships.

Let the value of αp at the periphery of the cell be $\alpha_p p$. Following the actual curve from the point $\alpha_p p$ to the left, we reach a point $\alpha p = \alpha_c p$ which represents the conditions in the centre of the cell. The position of $\alpha_c p$ depends partly on the dimensions of the cell and partly on the diffusion constant. Thus the dimensions of the cell and the diffusion constant may be of great significance for reactions at the centre, especially if $\alpha_c p$ lies at the steep part of a curve of type 1 or 3. It should be noted that a large change in q' sometimes corresponds to a large change in θ'/θ'' , i.e. a change in the ratio between the branch reaction rates. This fact should be considered in a discussion of cell division processes.

Let the point of intersection between curves 2 and 3 correspond to a point in a cell. When the conditions are slightly changed, e.g. a small increase of the cell or a small change in the diffusion constant, it is perhaps not important whether the point belongs to curve 2 or 3, but when the conditions are altered considerably it can be of great importance.

In cells corresponding to curves 1 and 3 a small change in $\alpha_p p$ alters the reaction rates only a little in the outer parts of the cell, but considerably in the inner parts, if $\alpha_c p$ is on the steep part of the curve. On the contrary, in cells corresponding to curve 2, a small change in $\alpha_p p$ alters the rates moderately over the whole cell. If the reaction rates in cells, for which curve 1 is valid, are altered by a change in the carrier chain, so that another curve is obtained, the alterations of the rates are distributed differently in the cell, depending on which of the R_k constants is changed.

Perhaps it may again be pointed out that each ϱ' value in Fig. 3 corresponds to a certain value of θ'/θ'' . This implies that not only ϱ' but also the ratio θ'/θ'' between the rates in the branches is changed, if we move along a curve or from one curve to another.

VII. Discussion of the ratio between the reaction rates in the branches. Changes in the common chain

When only the common chain is altered, it follows from p. 35, that the ratios between the rates ($\theta':\theta'': \dots$) vary with the respiration rate, if there is at least one carrier in any of the branches.

The special case that corresponds to (15b) (2 branches and one carrier in each) is now considered. If ϱ' is very small, θ'/θ'' is approximately equal to $R'_s/R''_s = E_1$. If the respiration velocity is altered by changes in the common chain, θ'/θ'' increases or decreases monotonously or is constant (cf. p. 36). When p and the R_k in the common chain $\rightarrow \infty$, θ'/θ'' thus tends to a limit, E_2 . E_1 and E_2 depend only on the R_k constants in the branches. (If p and the R_k constants in the common chain are very large, ϱ' is only limited by, and dependent on, the conditions in the branch.) The interval $E_1 \rightarrow E_2$ is the largest one within which θ'/θ'' can vary with unchanged branches. From (28a) and (15b) it follows that $E_2 = r'_1/r''_1$.

Consider Fig. 3, where each ϱ' corresponds to a certain value of θ'/θ'' . It is seen that if curve 1 or 2 represent the course of the reactions, θ'/θ'' has any value between E_1 and E_2 , as αp varies from $0 \rightarrow \infty$. For curve 2, however, larger values of αp are necessary to get θ'/θ'' values close to E_2 than for curve 1. If the conditions correspond to curve 3, on the contrary, θ'/θ'' never reaches the limit E_2 , however much αp may be increased, because R and thus also ϱ' are limited partly by R_1 .

It may be remarked that E_1 and E_2 , which depend only on R'_2 , R'_3 , R''_2 and R''_3 , as mentioned above, can be very different, so that one of them ≥ 1 and the other ≤ 1 . This implies that at a low respiration velocity, obtained by inhibition of the common chain, the rate in one of the branches dominates and at a large respiration velocity the rate of the other branch will dominate.

To demonstrate how the rates in the branches may vary, according to the formulae, a numerical example is given. It must be pointed out that the numerical values are in no way taken from biochemical experiments. In principle the values are possible, but it is not known, whether they correspond to any state in the living cell.

Numerical example: two branches, one carrier in each.

Let

$$\begin{array}{ll} R'_s = 10^3 & R''_s = 10^4 \\ R'_{s+1} = 1.2 \times 10^3 & R''_{s+1} = 500. \end{array}$$

Then $M'' = 19.17$, $r_1' = 545$, $r_1'' = 476$, $E_1 = 0.1$ and $E_2 = \frac{r_1'}{r_1''} = 1.14$.

Suppose that the common chain is inhibited, so that $q' = 200$, then

$$\frac{\theta'}{\theta''} = 0.48 \text{ (according to (15b))}, R = 617 \text{ and } q'' = 417.$$

Suppose on the other hand that the common chain is inhibited, so that $q' = 100$, then

$$\frac{\theta'}{\theta''} = 0.29, R = 445 \text{ and } q'' = 345.$$

Thus the ratios between q' in the different cases are $545:200:100 = 5.5:2:1$,
the ratios between q'' in the different cases are $476:417:345 = 1.4:1.2:1$,
the ratios between R in the different cases are $1021:617:445 = 2.3:1.4:1$,
and in the different cases θ'/θ'' is 1.14, 0.48, and 0.29.

This means that in the uninhibited case q' is 5.5 times larger than in the most inhibited case and larger than q'' , but q'' is only 1.4 times larger than in the most inhibited case. In the most inhibited case $q' < q''$.

VIII. Back reactions

The back reactions are now considered. It is, however, still assumed that no formation of free oxygen from the oxidized cytochrome oxidase takes place. Then

$$\begin{aligned} \theta' R = \theta' R_0 p x_0 = \theta' [R_1 (1 - x_0) x_1 - S_1 (1 - x_1) x_0] = \dots = R'_{g+1} (1 - x_g) x'_{g+1} - \\ - S'_{g+1} (1 - x'_{g+1}) x_g = \dots = R'_{s+1} (1 - x'_s) - S'_{s+1} x'_s \end{aligned} \quad (31)$$

and analogous equations for the other branches are valid. $S_1, S_2, \dots$ stand in the same relation to the back reactions as $R_1, R_2, \dots$ to the forward reactions, and thus $S_{s+1}^{(m)}$ is proportional to the concentration of the oxidized state of the substrate in the m th branch.

We consider only the ratios between the rates in the branches. Analogously to (4) we give the expression of x_g obtained from (31) for the different branches. Equation (5) is still valid. Only two special cases are treated.

2 branches, no carriers in the branches. ($s = g$)

$$x_g = \frac{R'_{s+1} - R \theta'}{R'_{s+1} + S'_{s+1}} = \frac{R''_{s+1} - R \theta''}{R''_{s+1} + S''_{s+1}}.$$

With (5) this gives

$$\frac{\theta'}{\theta''} = \frac{R'_{s+1} S''_{s+1} - R''_{s+1} S'_{s+1} + R (R'_{s+1} + S'_{s+1})}{-R'_{s+1} S''_{s+1} + R''_{s+1} S'_{s+1} + R (R''_{s+1} + S''_{s+1})}. \quad (32)$$

If $R'_{s+1}S''_{s+1} - R''_{s+1}S'_s = L < 0$ the substrate in the branch marked with a dash is reduced if $R < \frac{-L}{R'_{s+1} + S'_{s+1}}$. If $L > 0$ the substrate in the second branch is reduced if $R < \frac{L}{R''_{s+1} + S''_{s+1}}$.

2 branches, one carrier in each. ($s - g = 1$)

From (31) we obtain

$$x_g = \frac{R'_s R'_{s+1} - \theta' R (R'_s + R'_{s+1} + S'_{s+1})}{R'_s R'_{s+1} + S'_s S'_{s+1} - \theta' R (R'_s - S'_s)}$$

and an analogous expression for the other branch. Together with (5) these equations give the ratio between the rates. We shall only treat the case where S'_s and S''_{s+1} can be neglected but the back reactions between the substrates and the last carrier are considered. Then we obtain that θ'/θ'' corresponds to (15b) with R'_s replaced by

$$R'_s \frac{R'_{s+1}}{R'_{s+1} + S'_{s+1}}$$

and R''_{s+1} replaced by

$$R''_{s+1} \frac{R'_{s+1}}{R''_{s+1} + S''_{s+1}}.$$

IX. An application: A substrate oxidized in one of the branches is further oxidized in the other branch

(2 branches and one carrier in each)

Now a case is considered which illustrates the great significance of the value of θ'/θ'' , when the substrates in the two branches are two intermediates in the metabolism of a substance.¹

It is useful for the discussion to have an expression for θ'/θ'' where M'' from (13) is substituted in (15b)

$$\frac{\theta'}{\theta''} = \frac{\left(\frac{R''_s}{R''_{s+1}} - \frac{R'_s}{R'_{s+1}} \right) \theta' + R'_s}{R''_s} \quad (33)$$

We assume that there is a substrate, A' , in the branch marked with a dash and that its concentration is kept constant, i.e. R'_{s+1} is assumed to be constant. p and all the R_k except R'_{s+1} are also assumed to be constants. The oxidized state of A' , denoted A'' , is assumed to be oxidized in the other branch. At first $R''_{s+1} = 0$, but it

¹ The chain of enzyme reactions of a substance being metabolized is discussed by DIXON [2] as well as the possibility of making an intermediate substance accumulate by inhibition at one stage in the metabolism. Nevertheless it may be interesting to study the influence of the relationships between the branches of the respiration chain, when the substrates at the branches are intermediates in a metabolism.

increases during the experiment. (R'_{s+1} being proportional to the concentration of A'' .) When R'_{s+1} is very small, θ'/θ'' is very large (see (33)). As long as $\theta'/\theta'' > 1$, R'_{s+1} will increase. (More A'' is formed than is oxidized. It is assumed that the same quantity of oxygen is needed for oxidation of one mole of the two substrates.) θ'/θ'' , however, cannot be less than the initial value of

$$-\frac{R'_s}{R'_{s+1}} \varrho' + R'_s \frac{R'_s}{R''_s} = T, \quad (34)$$

however much R'_{s+1} may increase, as long as (15b) is valid, since the negative term is limited by the fact that ϱ' cannot increase. (15b) is valid only as long as the reduction of A'' to A' can be neglected. This is the case as long as $S'_{s+1} \ll R'_{s+1}$, i.e. at least when $a'_{s+1} \leq a'_{s+1}$, if the equilibrium of $A' \rightarrow A''$ is far to the right. The type of reaction at the steady state depends on the constants in the chains and on the equilibrium constant of the reaction $A' \rightarrow A''$. Some typical cases are considered.

(1) $R'_s < R''_s$.

The concentration a'_{s+1} increases and θ'/θ'' decreases. When a'_{s+1} becomes large enough to give θ'/θ'' the value 1, the steady state is obtained, M'' still being > 0 (see (33)). In this case it is reasonable to neglect the back reaction $A'' \rightarrow A'$ because a'_{s+1}/a'_{s+1} is not large, when $M'' > 0$.

(2) T in (34) > 1 at the beginning. Then R'_s must be $> R''_s$.

At first θ'/θ'' decreases rapidly and then more slowly, and A'' is accumulated. Gradually the concentration of A'' becomes so large that the velocity of $A'' \rightarrow A'$ becomes significant for the lowering of θ'/θ'' towards 1. But let us now assume that A'' can react according to another formula, $A'' \rightarrow F$. We assume that this reaction is so slow in comparison to the oxidation, that it can be neglected when the concentration of A'' is moderate, e.g. when $M'' = 0$ in the present case. When more A'' is formed, the reaction rate, f , of the reaction $A'' \rightarrow F$ increases at the same time as the rate of $A'' \rightarrow A'$. At the steady state $f = \varrho' - \varrho''$, and thus $\theta'/\theta'' > 1$. The relationship between the velocities of $A'' \rightarrow F$ and $A'' \rightarrow A'$ determines the ratio θ'/θ'' at steady state, i.e. how much the reaction $A'' \rightarrow F$ increases. An example is given below.

(3) *The intermediate cases, i.e. $R'_s > R''_s$ and T in (34) < 1 at the beginning.*

Whether the reactions (3) are similar to (1) or to (2) is dependent largely on the change of ϱ' during the reaction, and this depends not only on the constants in the branches, but on the constants of the common chain too.

Numerical examples

The examples are again theoretical and the comments on p. 41 are valid here too.

(a)—*Assumptions:* $R'_s = R''_s$. A quantity of A' is added to the carriers, and its concentration is kept constant. The oxidized state, A'' , is oxidized at the other branch and can moreover react according to $A'' \rightarrow F$ with the rate f . Furthermore it is assumed that at the steady state $S'_{s+1} = 0.01$, R'_{s+1} , $f = 0.001 \varrho'$ and $\varrho'/R'_{s+1} = 0.2$.

By using $q' = q'' + f$ at the steady state we obtain $\theta' / \theta'' = 1/0.999$. If R'_s in (33) is replaced by $R'_s \frac{R'_{s+1}}{R'_{s+1} + S'_{s+1}}$ (p. 43) it follows that $R''_{s-1} / R'_{s-1} = 0.957$.

(b)—Let us now take another example. We have the same conditions as above except that R''_s is lowered so that $R''_s = 0.8 R'_s$, i.e. a''_s is lowered, and since R'_{s+1} is also proportional to a''_s , R'_{s-1} is 0.8 times the value in (a) at the same value of a''_{s-1} . The difference in q' at the steady state between this case and case (a) depends on p and on the constants in the common chain. Suppose that q' lies between $0.16 R'_{s+1}$ and $0.2 R'_{s-1}$. The calculations are made under the assumption that the rate f of the reaction $A'' \rightarrow F$ is proportional to a''_{s+1} and then $f = \frac{R'_{s-1} \times 0.001 \times 0.2 \times R'_{s+1}}{R'_{s+1} \times 0.957 \times 0.8}$

according to the result in (a). If R'_s in (33) is replaced by $R'_s \frac{R'_{s+1}}{R'_{s+1} + S'_{s+1}}$ it follows that R'_{s+1} / R'_{s-1} lies between 5.5 and 3.8, the exact value being dependent on q' . This means that the rate of $A'' \rightarrow F$ is between 7.2 and 5.0 times larger than that in (a) but the oxidation rate is only partially changed.

(c)—Let us now take a third example. We have the same conditions in the branches as in (b) but we assume that it is possible to increase q' , so that $q' / R'_{s-1} = 0.3$ at the steady state, by changing p or the constants in the common chain. If we make similar calculations as in (a) and (b), we obtain $R''_{s-1} / R'_{s-1} = 2.0$ at the steady state. This means that the rate of the reaction of $A'' \rightarrow F$ is 2.6 times larger than that in (a).

Summary of results in (a), (b) and (c):

If q' / R'_{s+1}	0.20	0.16	0.20	0.30	and
R''_s / R'_s	1.0	0.8	0.8	0.8,	

the ratios between

the rates f are 1 : 7.2 : 5.0 : 2.6.

If $A'' \rightarrow F$ is a step in the formation of the carrier corresponding to R''_s or a substance that stimulates the common chain, $A'' \rightarrow F$ can be a regulation reaction.

X. Remarks

The carrier chains in the living cell are more complicated in several respects than the chains discussed. They have more carriers and more branches than the chains which have been treated in detail. Furthermore the branches themselves are probably likewise branched, so that there are several junctions, and in addition there exist carrier chains for radicals too, e.g. phosphate. Those at least for phosphate are coupled with the respiration carrier chains. The back reactions ought also to be considered. All these facts make the relationships very complicated. Nevertheless, a schematic calculation such as the one given above can be valuable for obtaining an idea of the properties of the carrier chains and of their significance for the regulation of the reactions.

Even in the simple cases considered here it is difficult to get a clear view of the relationships, because there are so many undetermined constants. For that reason some numerical examples have been given. It should be pointed out, however, that

they are not taken from experiments. The justification for giving them is that they show, in extreme cases, the influence on the metabolism due to branched carrier chains in comparison with single enzymes. Perhaps the constants do not correspond to the conditions in ordinary cells. It is, however, at least possible that they correspond to conditions in disturbed or abnormal cells. Sometimes experiments may occur at abnormal conditions, and it would then be necessary to know how the relationships which depend on the chains vary with the conditions. If the formulae are applied to other carrier processes, the examples may be significant.

A characteristic of the chains is that the ratios between the rates in the branches depend on the constants in the common chain and not only on the constants in the branches. The examples on p. 44 show how changes in these ratios can be very significant, when the substrates are intermediates in the metabolism of a substance, and thus they show the significance of p and the constants in the common chain. They show e.g. that a decrease of the O_2 pressure may cause an accumulation of an intermediate substance and thus an increase in the reactions $A'' \rightarrow F$. These facts emphasize the significance of the discussion of the rates and conditions at different distances from the periphery (p. 42).

ACKNOWLEDGEMENT

I am much indebted to Professor O. KLEIN and Professor J. RUNNSTRÖM for their suggesting a continuation of their investigation, which has been the basis for this paper, and for their stimulating interest and advice. I should like to express my thanks to Dr. INGA HJALMARS for her unfailing support and interest and to Fil. lic. L. ERNSTER and Fil. lic. L. TREFFENBERG for stimulating discussions. This work was made possible by a grant from *Statens Naturvetenskapliga Forskningsråd*.

Institute of Theoretical Physics and The Wenner-Gren Institute for Exp. Biology, University of Stockholm, May 1955.

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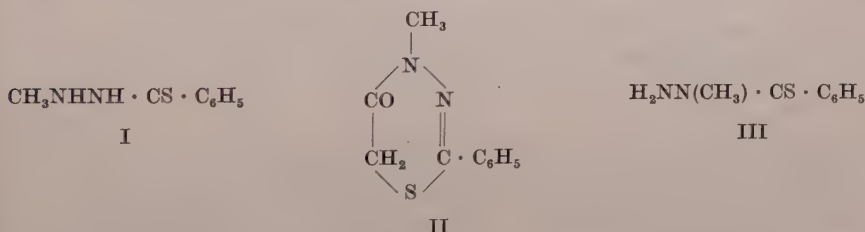
Versuche mit Methyl- und Methylthiobenzhydraziden

Von BROR HOLMBERG

1. Einleitung und Übersicht

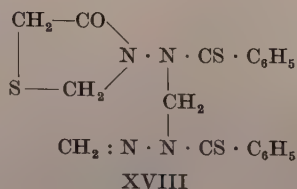
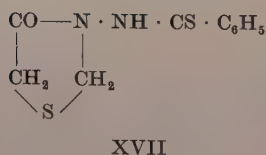
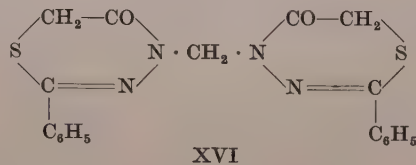
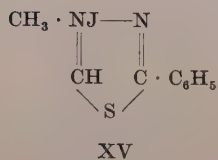
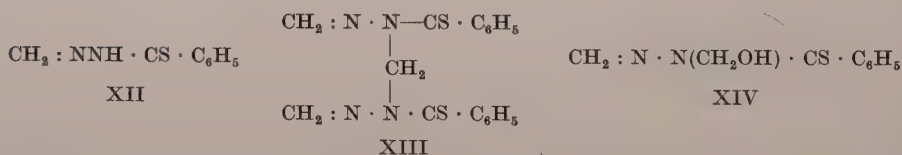
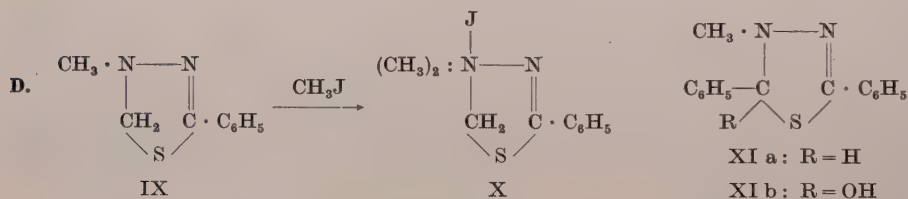
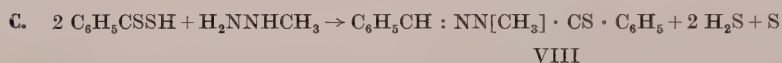
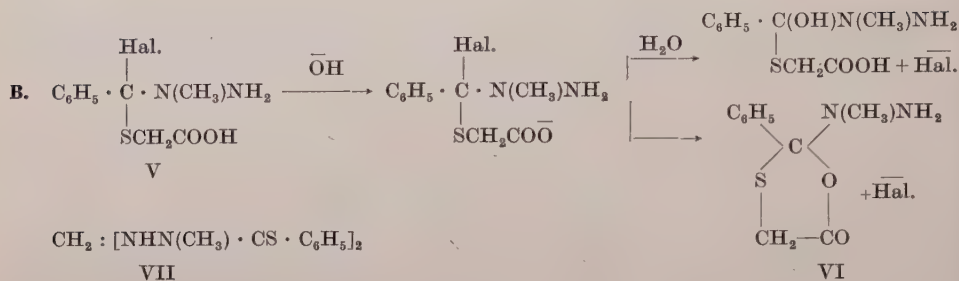
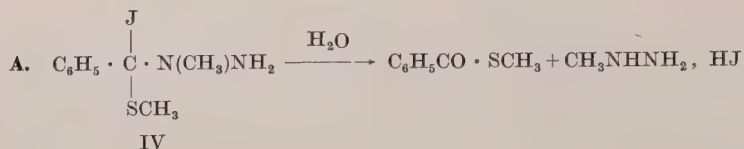
Bei der Einwirkung von Ammoniak auf ein aus dem *l*-Arabinose-methylen-thiobenzhydrazid in salzsaurer Lösung entstandenes Umlagerungsprodukt erhielt der Verf. (1, S. 105) eine ölige Base, welche ein Hydrochlorid vom Schmp. 169°–171° und der Zusammensetzung $C_8H_{11}N_2ClS$ und ein analog zusammengesetztes Hydrojodid vom Schmp. 146°–148° lieferte. Die Base wurde von Natronlauge gelöst, und beim Versetzen der Lösung mit Monobromazetat und nachfolgendem Ansäuern mit Essigsäure wurde eine Verbindung vom Schmp. 90°–91° und der Zusammensetzung $C_{10}H_{10}ON_2S$ erhalten. Da das Thiobenzhydrazid selber in der gleichen Weise ein als 2-Phenyl-5-oxo-5,6-dihydro-1,3,4-thiopyridazin formuliertes Produkt lieferte (2), wurde die aus dem genannten Arabinose-Derivate gewonnene Base als 2-Methylthiobenzhydrazid bzw. 1-Methyl-2-thiobenzoylhydrazin (I) und ihr Umsetzungsprodukt mit dem Bromazetat als 4-Methyl-2-phenyl-5-oxo-5,6-dihydro-1,3,4-thiopyridazin (II) formuliert.

Später ausgeführte Versuche, das 2-Methylthiobenzhydrazid durch Umsetzen der Thiobenzoylthioglykolsäure mit Methylhydrazin darzustellen, gelangen indessen nicht, sondern hierbei entstand eine kristallisierte Verbindung vom Schmp. 91°–92°, welche nicht alkalilöslich war und deshalb als das mit der obigen Base isomere 1-Methylthiobenzhydrazid (III) aufgefasst werden muss.¹ Ein Hydrochlorid dieser Base, welches ihrer leichten Hydrolysierbarkeit wegen nicht ganz rein erhalten werden konnte, schmolz bei 125°–132°, und beim Umsetzen der Base mit Methyljodid in Äthylazetat entstand ein Additionsprodukt vom Schmp. 139°–141° und der Zusammensetzung $C_9H_{13}N_2JS$, welches beim Erhitzen in wässriger Lösung in Methyl-



¹ In analoger Weise erhielten JENSEN und MIQUEL (3) ein Produkt vom Schmp. 88°, welches sie indessen als 2-Methylthiobenzhydrazid auffassten, und mit dem sie ein Nickelsalz der Zusammensetzung $Ni(C_8H_9N_2S)_2$ darstellen konnten.

B. HOLMBERG, *Methyl- und Methylthiobenzhydrazide*



thiobenzoat und jodwasserstoffsäures Methylhydrazin zerfiel (A), weshalb es analog einem von WALLACH (4) aus Thioazetanilid und Äthylbromid erhaltenen Additionsprodukte¹ formuliert werden kann (IV). Auch mit den *Monobrom-* und *Monojodessigsäuren* wurden analoge Additionsprodukte des Schmp. 113°–114° bzw. 123°–124° erhalten (V). Bei den Titrierungen mit Natronlauge verbrauchten diese Verbindungen etwas mehr Lauge, als nur die Carboxylgruppen erfordern, was auf eine gleichzeitig mit der Neutralisierung eingetretenen Abspaltung der Halogene beruhen dürfte. Die Lösungen wurden jedoch allmählich wieder sauer, und da die Säureproduktion in den nach gleichen Zeiten aufs neue neutralisierten Lösungen in beiden Fällen ungefähr gleich schnell verlief, dürfte gemäss (B) und in Konkurrenz mit dem Austausch der Halogene gegen Hydroxyl auch die Bildung eines unbeständigen Oxthiophanons (VI) eingetreten sein. — Mit *Formaldehyd* lieferte das 1-Methylthiobenzhydrazid ein *bis*-Derivat (VII), und mit der *l-Arabinose* setzte es sich zu einem Thiohydrazid bzw. Thiohydrazon (vgl. 1) um, welches ein Mol H₂O enthält und in wasserfreier Form bei 121°–122° schmolz und in Pyridin $[\alpha]_D^{20} = +16,8^\circ$, $[M]_D^{20} = +50,1^\circ$ zeigte. — Mit *Benzaldehyd* kondensierte es sich zu einem Thiohydrazon (VIII) vom Schmp. 126°–127,5°, welches schon früher WUYTS und LI CHIA KUANG (6) durch Umsetzung des Methylhydrazins mit der Dithiobenzoessäure gemäss der Bruttogleichung (C) erhalten haben, und für welches sie den Schmp. 124°–125° fanden.

Einige der mit dem 1-Methylthiobenzhydrazid durchgeführten Reaktionen wurden auch bei dem 2-Isomeren geprüft. Hierbei wurde in anspruchloser Ausbeute eine Additionsverbindung mit dem *Methyljodid* vom Schmp. 123°–125° erhalten, welche bei der Titrierung mit Natronlauge eine nach Methylmercaptan riechende Lösung lieferte und also mit dem aus dem 1-Isomeren in der gleichen Weise erhaltenen Produkte übereinstimmend konstituiert sein dürfte. — Mit *Formaldehyd* lieferte das 2-Methylthiobenzhydrazid ein öliges Produkt, welchem die Konstitution eines 4-Methyl-2-phenyl-1,3,4-thiodiazolins (IX) zukommen dürfte. Mit Methyljodid lieferte diese Verbindung gemäss (D) ein Additionsprodukt C₁₀H₁₃N₂JS vom Schmp. 192°–193°, welches als ein 4,4-Dimethyl-2-phenyl-1,3,4-thiodiazoliniumjodid (X) anzusehen ist. — Mit der *l-Arabinose* ergab dasselbe Thiohydrazid ein Produkt vom Schmp. 142°–148° und in Pyridin von $[\alpha]_D^{20} = \frac{5 \text{ Min.}}{-53,7^\circ}$, $\frac{25 \text{ Min.}}{-106,3^\circ}$, welches nach der Umkristallisierung aus Alkohol den Schmp. 140°–146° und $[\alpha]_D^{20} = \frac{6 \text{ Min.}}{-112,2^\circ}$, $\frac{20 \text{ Min.}}{-105,1^\circ}$ zeigte, weshalb die Präparate, wie die Glykothiodiazoline von WUYTS (7) (siehe auch bei HOLMBERG [8]), als Gemische aus leicht ineinander übergehenden Stereoisomeren anzusehen sind. — Aus dem 2-Methylthiobenzhydrazid und *Benzaldehyd* wurde das 4-Methyl-2,5-diphenyl-1,3,4-thiodiazolin (XI a) erhalten, welches bei 63°–65° schmolz. Beim Erhitzen an der Luft nahm diese Verbindung Sauerstoff auf und ging in eine Verbindung vom Schmp. 156°–157° über. Dieselbe Verbindung wurde durch Behandlung des Methylthiobenzthiodiazolins mit Pyridiniumtribromid und nachfolgende Verseifung des Bromierungsproduktes mit Natronlauge erhalten, und sie wird daher als 4-Methyl-2,5-diphenyl-5-oxo-1,3,4-thiodiazolin (XI b) aufgefasst.

Versuche durch Umsetzung des *Thiobenzhydrazids* selber mit Methyljodid ein Methylderivat darzustellen gelangen nicht, denn in alkalischer Lösung entstand hier, wie bei einem früheren Versuche mit Benzylbromid (2) nur das 3,6-Diphenyl-1,4-dihydro-1,2,4,5-tetrazin als erfassbares Produkt, und aus den Komponenten allein

¹ Betreffs weiterer Literatur über Umsetzungen von Thiocarbonylverbindungen mit Haloidestern siehe bei KNORR (5).

in Äthylazetat wurden 2,5-Diphenyl-1,3,4-thiodiazol nebst jodwasserstoffsäurem Hydrazin und Schwefelwasserstoff bzw. mehr oder weniger hoch methylierte Derivate davon gebildet.

Das aus dem 2-Methylthiobenzhydrazid mit Formaldehyd usw. erhaltene $C_{10}H_{13}N_2JS$ -Produkt wurde auch aus den vom Verfasser früher (2 und 1, S. 97) beschriebenen *Methylthiobenzhydraziden* (XII–XIV) und Methyljodid erhalten. — Dass das *Monomethylthiobenzhydrazid* bei Behandlungen mit schwachen Oxydationsmitteln 2 Atome Wasserstoff verliert und in das 2-Phenyl-1,3,4-thiodiazol übergeht, wird in einem anderen Zusammenhang vom Verf. mitgeteilt (9), und als jetzt diese Verbindung mit *Methyljodid* behandelt wurde, entstand eine Additionsverbindung vom Schmp. 181° – 183° , welcher die Konstitution des 4-Methyl-2-phenyl-1,3,4-thiodiazoliumjodids¹ (XV) zukommen dürfte. Die entsprechende, freie Base schmolz bei 75° – 77° , lieferte mit Salzsäure ein Chlorid vom Schmp. 168° – 170° und war auch in Natronlauge löslich. Als eine Lösung des Methylthiobenzhydrazids in Natronlauge mit *Monobromazetat* versetzt wurde, wurde hauptsächlich das Methylen-bis-methylthiobenzhydrazid gebildet, aber in kleiner Menge entstand auch eine Verbindung vom Schmp. 183° – 184° und der Zusammensetzung $C_{19}H_{16}O_2N_4S_2$, was mit der Konstitution des 4,4'-Methylen-bis-5,6-dihydro-2-phenyl-5-oxo-1,3,4-thiadiazins (XVI) übereinstimmt. — Als schliesslich das chlorwasserstoffsäure Methylthiobenzhydrazid mit *Thioglykolsäure* in etwas Wasser behandelt wurde, entstand unter Abspaltung von Wasser aus dem primär gebildeten Additionsprodukte eine Verbindung vom Schmp. 103° – 104° und der Konstitution des 3-Thiobenzoylamino-4-oxo-1,3-thiazolidins (XVII).

Das *Methylen-bis-methylthiobenzhydrazid* lieferte bei den Behandlungen mit *Pyridiniumtribromid*, mit *Methyljodid* und mit *Thioglykolsäure* dieselben Produkte wie das *Monomethylthiobenzhydrazid*. Im letzteren Falle wurde indessen auch eine Verbindung vom Schmp. 103° – 105° und der Zusammensetzung $C_{19}H_{18}ON_4S_3$ erhalten, welche als ein durch Wasserabspaltung ringgeschlossenes Additionsprodukt der Ausgangssubstanzen (XVIII) formuliert werden kann.

Das *Methylen-oxymethylthiobenzhydrazid*, welches zuerst bei Einwirkungen des Formaldehyds auf Zuckerthiobenzhydrazide in alkalischen Lösungen gewonnen wurde (1), wurde jetzt direkt aus Thiobenzhydrazid und Formaldehyd in Natronlauge dargestellt. Es geht indessen so leicht in das Methylen-bis-methylthiobenzhydrazid über, dass keine eigenen Derivate davon erhältlich sind, und es ist daher begreiflich, dass es mit *Methyljodid* dasselbe $C_{10}H_{13}N_2JS$ -Produkt wie die beiden anderen Methylthiobenzhydrazide liefert.

Diese Untersuchung wurde in der damaligen org.-chemischen Abteilung des Nobelinstitutes der Kgl. Akademie der Wissenschaften, Stockholm, begonnen und in der entsprechenden Abteilung des Universitätslaboratoriums in Lund abgeschlossen. — Die Elementaranalysen wurden von Frau MAJBRYTT VESTERGAARD in dem genannten Nobelinstitute ausgeführt.

2. Versuche mit dem 1-Methylthiobenzhydrazid

Als eine Lösung von 21 g Thiobenzoylthioglykolsäure in 100 ml 1-n. Natronlauge mit einer aus 14,5 g gemäss THIELE (10) und *Organic Syntheses* (11) hergestelltem, schwefelsäurem Methylhydrazin, 8 g Natriumhydroxyd und 50 ml Wasser bereiteten

¹ Bei der Behandlung des 2,5-Diphenyl-1,3,4-thiodiazols mit Methyljodid trat keine Umsetzung ein.

Lösung versetzt wurde, trat sogleich Emulgierung ein und ein Kristallpulver wurde abgeschieden, welches am folgenden Tage abgenutscht und an der Luft getrocknet wurde, wonach es 14,5 g wog und bei 87° – 89° schmolz. Bei der Umkristallisierung aus 60 ml gew. Alkohol wurden hieraus 9,6 g 1-Methylthiobenzhydrazid (III) als schwach cremefarbene, flache Prismen vom Schmp. 91° – 92° erhalten.

Für $C_8H_{10}N_2S$ (166,24):

ber.: C 57,80, H 6,06, N 16,85, S 19,29 %.

gef.: C 57,81, H 6,06, N 16,82, S 19,17 %.

Das 1-Methylthiobenzhydrazid wurde nicht merkbar von 1-*n*. Natronlauge oder 1-*n*. Salzsäure gelöst, aber als eine warme Lösung von 1,7 g des Hydrazids in 10 ml Alkohol mit 10 ml 5-*n*. Salzsäure versetzt wurde, entstand ein homogenes Gemisch, welches beim Erkalten nicht verändert wurde und dann bei der freiwilligen Eindunstung zu 8 g Gewicht 1,3 g auf Tonscherben getrocknete, blassgelbe, schmale Tafelchen lieferte. Dieses Produkt schmolz unter schwacher Gasentwicklung bei 125° – 132° und bestand gemäss Analyse aus dem nicht ganz reinen Hydrochlorid des 1-Methylthiobenzhydrazids.

Für $C_8H_{11}N_2ClS$ (202,71):

ber.: Cl 17,49 %, Äquiv.-Gew. 202,7,

gef.: Cl 16,99 %, Äquiv.-Gew. 205,8.

Bei der Behandlung einer Probe des Salzes mit Wasser wurde unmittelbar die freie Base als fast rein weisses Kristallpulver vom Schmp. 89° – 91° abgeschieden.

Beim Versetzen einer Lösung von 1,7 g 1-Methylthiobenzhydrazid in 20 ml Äthylazetat mit 6 g Methyljodid entstand ein homogenes Gemisch, welches nach einigen Minuten sich zu trüben begann und im Laufe einiger Tage 3,4 g farblose, schmale Tafelchen oder dünne Prismen vom Schmp. 136° – 138° ausschied. Das Produkt enthielt 40,51 % J, und bei schnellem Umkristallisieren von 2,9 g davon aus 10 ml gew. Alkohol wurden 1,1 g Kristalle wie vorher, vom Schmp. 139° – 141° und der Zusammensetzung eines Additionsproduktes des 1-Methylthiobenzhydrazids mit dem Methyljodid (IV) erhalten.

Für $C_8H_{13}N_2JS$ (308,20):

ber.: C 35,07, H 4,25, N 9,09, J 41,18, S 10,40 %,

gef.: C 35,10, H 4,25, N 9,07, J 41,01, S 10,35 %.

Eine Lösung von 0,1808 g des analysierten Jodids in 10 ml Wasser verbrauchte (Phenolphthalein) zuerst und mit schnell verblussendem Umschlag 0,60 ml 0,0961-*n*. Natronlauge und nach Zusätzen von $4 \times 1,00$ ml der Lauge traten neue Entfärbungen nach 0,5, 2,5, 7 und 14 Minuten ein. Die Lösung, welche dann nach Methylmercaptan roch, wurde mit noch 2,00 ml der Lauge versetzt, wonach sie am folgenden Tage von 0,46 ml 0,1011-*n*. Salzsäure entfärbt wurde, was dem Äquiv.-Gew. 307, ber. 308,2, entspricht. Beim Titrieren mit 1/10-*n*. Jodlösung verbrauchte sie dann 4,5 ml davon unter Abscheidung von ein wenig eines braunschwarzen Pulvers.

Als 3,1 g des rohen Jodids mit 30 ml Wasser auf dem Wasserbade erhitzt wurden, trat bald Emulgierung ein, und nach einer Stunde waren 1,3 g eines Öles abgeschieden, welches nach dem Waschen mit Wasser und Trocknen mit geschmolzenem Chlorcalcium gemäss der Analyse aus etwas unreinem Methylthiobenzoat bestand.

Für C_8H_8OS (152,21):

ber.: C 63,12, H 5,30, S 21,07 %.

gef.: C 62,19, H 5,15, S 20,83 %.

Beim Erhitzen auf dem Wasserbade von 0,9 g des analysierten Esters mit 10 ml Alkohol und 20 ml 1-n. Natronlauge entstand bald eine klare Lösung, welche nach einer Stunde, Verjagen des Alkohols und Ansäuern mit Salzsäure stark nach Methylmercaptan roch und 0,65 g Benzoesäure vom Schmp. 122° – 123° und Äquiv.-Gew. 122,8, ber. 122,1, ausschied. — Die von dem Methylthiobenzoat oben abseparierte Wasserlösung wurde bei fortgesetztem Erhitzen während einer Stunde nur schwach milchig und beim Erkalten schied sie dann eine Spur eines Öles ab, welches bei freiwilliger Eindunstung des Gemisches vermehrt wurde und allmählich teilweise kristallisierte. Hierbei wurden flache Prismen erhalten, welche nach dem Trocknen auf Tonscherben 1,0 g wogen, bei 81° – 83° schmolzen und gemäss der Analyse aus dem fast reinen Hydrojodid des Methylhydrazins bestanden.

Für CH_7N_2J (174,00):

ber.: C 6,90, H 4,06, N 16,10, J 72,94 %, Äquiv.-Gew. 174,0,

gef.: C 7,24, H 4,05, N 15,97, J 73,39 %, Äquiv.-Gew. 172.¹

Als eine Lösung von 0,35 g des analysierten Produktes in 2 ml Wasser mit einer Lösung von 0,5 g Thiobenzoylthioglykolsäure in 4,7 ml 1-n. Natronlauge versetzt wurde, entstand sogleich eine Emulsion, welche 0,30 g 1-Methylthiobenzhydrazid als grobkristallinisches Pulver vom Schmp. 88° – 89° , in Gemisch mit einer authentischen Probe $88,5^{\circ}$ – 90° , absetzte.

Bei einer unvorsichtigen Umkristallisierung von 3 g des Jodids (IV) aus 10 ml gew. Alkohol wurden 0,8 g Kristalle vom Schmp. 139° – 141° erhalten, und als die Mutterlauge mit 25 ml Wasser versetzt wurde, entstand eine Emulsion, welche 0,7 g gelbrotes Öl ausschied. Auch dieses Öl bestand aus Methylthiobenzoat, denn als es mit etwas Hydrazinhydrat versetzt wurde, trat schwache Wärmeentwicklung ein und eine Lösung entstand, welche 0,4 g Benzhydrazid als farblose Nadeln vom Schmp. 113° – 114° ² und mit 20,60, ber. 20,58, % N ausschied. — Bei der Behandlung von 2,7 g desselben Jodids mit 10 ml konz. Ammoniak trat unmittelbar Abscheidung eines Öles ein, welches jedoch allmählich fast vollständig in Lösung ging. Die nach Methylmercaptan riechende Lösung lieferte dann bei freiwilliger Eindunstung 1,4 g auf Tonscherben getrocknete Täfelchen vom Schmp. 148° – 155° , nach der Umkristallisierung aus wenig Wasser 160° – 161° und dann von der Zusammensetzung eines jodwasserstoffsäuren N-Methylbenzhydrazidins.

Für $C_8H_{12}N_2J$ (277,12):

ber.: C 34,67, H 4,37, N 15,16, J 45,80 %,

gef.: C 34,51, H 4,43, N 15,10, J 45,92 %.

Aus 1,7 g 1-Methylthiobenzhydrazid, 4 g *Monobromessigsäure* und 100 ml abs. Äther wurde ein homogenes Gemisch erhalten, welches im Laufe von 2 Tagen 0,4 g einer kristallinischen Bodenkruste absetzte. Die davon abdekantierte Lösung schied

¹ Phenolphthalein.

² Für dieses Hydrazid hat der Verf. früher (12) den Schmp. 114° – 115° gefunden.

dann während 12 Tage 1,3 g farblose Kristalle vom Schmp. 113°–114° (unter Gasentwicklung) aus, welche die dem Methyljodid-Produkt (IV) analoge Zusammensetzung zeigte.

Für $C_{10}H_{13}O_2N_2BrS$ (305,20):

ber.: C 39,35, H 4,29, N 9,18, Br 26,18, S 10,51 %,

gef.: C 39,26, H 4,35, N 9,21, Br 26,28, S 10,55 %.

Eine Lösung von 0,1638 g des Produktes in 16 ml Wasser ergab 5 Minuten nach dem Zusatz des Wassers mit Phenolphthalein und 6,10 ml 0,0949-n. Natronlauge eine schnell verblassende Rotfärbung, Äquiv.-Gew. 283, ber. 305; nach Zusätzen von $4 \times 1,00$ ml derselben Lauge verblieben die Färbungen 6, 12, 26 und 60 Minuten sichtbar, aber nach dem Zusatz von noch 1,00 ml der Lauge wurde die Lösung am folgenden Tage durch 0,20 ml 0,1011-n. Salzsäure entfärbt, Äquiv.-Gew. also 156, ber. 152,6. — Bei einem neuen Versuch mit 0,1530 g Sbst. in 20 ml Wasser verbrauchte die Lösung 60 Minuten nach dem Zusatz des Wassers 6,25 ml der Lauge, Äquiv.-Gew. 258, und dann in Intervallen von je ΔT Stunden bei gew. Temperatur:

ΔT :	1	48	48	48	72	72	96
Lauge, ml:	0,90	2,50	0,45	0,35	0,20	0,20	0,15
ΔT :	192	144	336	1 ¹	168	2 ²	336
Lauge, ml:	0,10	0,10	0,15	0,20	0,10	0,20	0,10

Insgesamt wurden also 11,95 ml der Lauge verbraucht, was dem Äquiv.-Gew. 135, ber. 152,6, entspricht.

Als eine Lösung von 2,3 g 1-Methylthiobenzhydrazid in 25 ml Äthylazetat mit 5 g *Monojodessigsäure* versetzt wurde, entstand ein homogenes Gemisch, welches sich bald trübte und eine klebrige Masse absetzte. Nach einer halben Stunde wurde die darüber stehende Lösung abdekantiert, wonach die Masse bis zum folgenden Tage in 1,3 g einer gelbbraunen, etwas teigigen Masse überging. Die abdekantierte Lösung wurde mit bei einem Vorversuch erhaltenen Kristallen geimpft, wonach schnell Kristallisierung begann und am folgenden Tage wurden 3,0 g mit Essigester gewaschene, crèmefarbene, dünne Prismen vom Schmp. 123°–124° erhalten.

Für $C_{10}H_{13}O_2N_2JS$ (352,21):

ber.: C 34,10, H 3,72, N 7,95, J 36,04, S 9,10 %,

gef.: C 34,11, H 3,72, N 8,01, J 36,15, S 9,06 %.

Eine Lösung von 0,1773 g des analysierten Produktes in 20 ml Wasser verbrauchte 2 Minuten nach dem Zusatz des Wassers 6,20 ml 0,0949-n. Natronlauge, Äquiv.-Gew. 301, ber. 352,2; die Färbung verschwand schnell, und nach den Zusätzen von $2 \times 1,00$ ml der Lauge war die Rotfärbung 10 bzw. 18 Minuten sichtbar. Nach dem Zusatz von noch 5,00 ml der Lauge wurde die nach 2 Tagen immer noch rote Lösung durch 2,40 ml 0,1011-n. Salzsäure entfärbt, Äquiv.-Gew. 175,6, ber. 176,1. — Eine Lösung von 0,1779 g Sbst. in 20 ml Wasser verbrauchte wie oben, aber eine Stunde nach dem Zusatz des Wassers, 6,30 ml der Lauge, Äquiv.-Gew. 298, und dann wie bei dem Bromprodukt oben:

¹ Nach 1 Stunde auf dem Wasserbade.

² Nach 2 Stunden auf dem Wasserbade.

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ΔT :	1	48	48	48	72	72	96
Lauge, ml:	0,70	3,00	0,15	0,15	0,07	0,07	0,08
ΔT :	192	144	336	1 ¹	168	2 ²	336
Lauge, ml:	0,05	0,10	0,15	0,20	0,10	0,25	0,05

Laugenverbrauch insgesamt 11,42 ml und Äquiv.-Gew. 164, ber. 176,1.

Als 2,0 g des Jodessigsäure-Produktes mit 20 ml Wasser behandelt wurden, entstand eine klebrige Masse, welche nach 2 Tagen aus einer öligen Kristallmasse bestand. Auf Tonscherben lieferte diese 0,9 g Kristalle, welche bei der Umkristallisierung aus Benzol fast ganz reine Benzoylthioglykolsäure als glasklare, dünne Prismen oder schmale Täfelchen vom Schmp. 104°–106°³ lieferte.

Für $C_9H_8O_3S$ (196,22):

ber.: C 55,09, H 4,11, S 16,34 %,

gef.: C 55,55, H 4,28, S 16,19 %.

Ein Gemisch aus 3,5 g desselben Produktes mit 10 ml Wasser lieferte mit 17,6 ml 1-n. Natronlauge und Phenolphthalein eine rosafarbene, trübe Lösung, welche sich bald entfärbte und dann nach Zusätzen von 1,00, 1,4 bzw. 1,0 ml der Lauge diese in 8, 15 bzw. 25 Minuten verbrauchte. Nach Zusatz von noch 1,0 ml der Lauge wurden 2 Tage später beim Filtrieren des Gemisches 0,4 g Kristallpulver erhalten, welches bei 94°–96° schmolz und der Analyse gemäss als etwas unreines 1,4-Dimethyl-2,5-diphenyl-1,4-dihydro-1,3,4,6-tetrazin anzusehen ist.

Für $C_{16}H_{16}N_4$ (264,32):

ber.: C 72,70, H 6,10, N 21,20, S 0,00 %,

gef.: C 72,23, H 6,14, N 20,80, S 0,30 %.

Das Filtrat wurde beim Anzäuern mit 5 ml 5-n. Salzsäure nicht getrübt und lieferte dann nach dreimaligen Ausschütteln mit 50 ml Äther Säuren entsprechend 10,0 + 3,5 + 1,5 = 15,0 ml 1-n. Natronlauge. Der Äther ergab dann bei freiwilliger Eindunstung nur 0,05 g eines viskosen Öles; da die erhaltene, neutrale Wasserlösung von Salzsäure nur schwach emulgiert wurde, dürften die anderen Zersetzungsprodukte des Ausgangsmateriales hauptsächlich aus Thioglykolsäure und Jodwasserstoff bestanden haben.

Nach dem Versetzen einer Lösung von 3,4 g 1-Methylthiobenzhydrazid in 50 ml Alkohol mit 2 g *Formalin* schied das Gemisch bis zum folgenden Tage 0,8 g schwach gelbliche, schmale Prismen vom Schmp. 129°–131° aus, welche die Zusammensetzung des Methylene-bis-methylthiobenzhydrazids (VII) zeigten.

Für $C_{17}H_{20}N_4S_2$ (344,49):

ber.: C 59,27, H 5,85, N 16,26, S 18,62 %,

gef.: C 59,04, H 5,77, N 16,22, S 18,50 %.

Die Mutterlauge des analysierten Produktes wurde beim Absaugen emulgiert und schied bei freiwilliger Eindunstung eine klebrige Masse aus, welche bei Behandlungen mit Salzsäure, Natronlauge usw. nichts Kristallisierbares lieferte.

¹ Nach 1 Stunde auf dem Wasserbade.

² Nach 2 Stunden auf dem Wasserbade.

³ Für reine Benzoylthioglykolsäure hat der Verf. (12) den Schmp. 107°–108° angegeben.

Beim Versetzen einer Lösung von 6,6 g 1-Methylthiobenzhydrazid in 80 ml Alkohol mit einer Lösung von 6 g *l*-Arabinose in 20 ml 2-*n*. Salzsäure entstand ein homogenes Gemisch, welches sich schwach erwärmte und nach einer Stunde von 44 ml 1-*n*. Natronlauge neutralisiert (Lackmus) wurde, wonach es beim Eindunsten in einem Luftstrom zu 67 g Gewicht 8 g mit 20 ml Wasser gewaschenes Kristallpulver (Präp. 1) absetzte. In zugeschmolzenem Röhrchen schmolz dieses Produkt bei 84°–87° und im Vakuum über Phosphorsäureanhydrid verlor es 10,10, für 2 H₂O ber. 10,77, % am Gewicht, wonach es bei ca. 115°–118° schmolz. Beim Umkristallisieren von 3 g des Produktes aus 10 ml gew. Alkohol wurden im Eisschrank und nach Impfen mit dem Rohprodukte 1,8 g flache Prismen vom Schmp. (wie oben) 93°–95°, mit 10,65 % H₂O und in trockener Form vom Schmp. 121°–122° erhalten (Präp. 2). Die getrockneten Proben ergaben bei den Analysen:

Für C₁₃H₁₈O₄N₂S (298,36):

ber.: C 52,33, H 6,08, N 9,39, S 10,75 %,

für Präp. 1 gef.: C 52,64, H 6,05, N 10,09, S 11,31 %,

für Präp. 2 gef.: C 52,15, H 6,03, N 9,40, S 10,74 %.

Eine Lösung von 0,4143 g des getrockneten Präp. 1 in Pyridin auf 10,00 ml Volumen gelöst zeigte 10 und 60 Minuten nach dem Zusatz des Lösungsmittels $\alpha_D^{20} = +0,70^\circ$, $[\alpha]_D^{20} = -16,9^\circ$, während das Präp. 2 in der gleichen Weise $[\alpha]_D^{20} = +16,8^\circ$, $[M]_D^{20} = +50,1^\circ$ ergab.

Bei schnellem Erhitzen von 2,0 g des rohen *l*-Arabinose-1-methylthiobenzhydrazids mit 20 ml Wasser entstand eine gelbgrüne Lösung, welche beim Erkalten und bis zum folgenden Tage 1,2 g fast unverändertes Material vom Schmp. (wie oben) 86°–89°, mit 9,89 % H₂O und in trockener Form vom Schmp. 112°–115° und $[\alpha]_D^{20} = -16,3^\circ$ (in Pyridin) ausschied. — Bei einem Parallelversuch wurde die Lösung 4 Stunden auf dem Wasserbade erhitzt, wobei sie ein braunschwarzes Öl ausschied, welches beim Erkalten kristallinisch erstarrte und nach der Aufnahme mit Äther und freiwilligem Verdunsten dieses 1,0 g wog und bei ca. 80°–84° schmolz. Nach der Umkristallisierung aus Alkohol bestand das Produkt aus cremefarbenen, flachen Prismen vom Schmp. des 1-Methylthiobenzhydrazids, 89°–91°. Die mit Äther extrahierte Reaktionslösung schied bei der freiwilligen Eindunstung nur eine unbedeutende Menge klebriger Massen ab, und nach dem Verdünnen des Filtrates davon mit 10 ml Wasser und Zugabe von 1,5 g Thiobenzhydrazid in 10 ml Alkohol schied das homogene Gemisch bis zum folgenden Tage 0,4 g des vom Verfasser früher (1) beschriebenen *l*-Arabinose-thiobenzhydrazids als haarfeine Nadeln vom Schmp. 165°–166° (Zersetzung) und $[\alpha]_D^{20} = -35,0^\circ$ (Pyridin) aus. Die Mutterlauge ergab bei freiwilliger Eindunstung 1,6 g einer teigigen Kristallmasse, welche beim Behandeln mit Benzol ein ungelöstes Pulver vom Schmp. 148°–152° lieferte. Nach der Umkristallisierung dieses Produktes aus Alkohol schmolz es bei 166°–167,5° (Zersetzung) und enthielt 11,30, für das Arabinose-thiobenzhydrazid ber. 11,28, % S.

Nach 2-stündigem Schütteln von 3,0 g *l*-Arabinose-1-methylthiobenzhydrazid mit 40 ml 0,5-*n*. Natronlauge wurden beim Absaugen 1,45 g eines Kristallpulvers erhalten, welches aus freigemachtem 1-Methylthiobenzhydrazid vom Schmp. 88°–90° bestand.

Für C₈H₁₀N₂S (166,24):

ber.: C 57,80, H 6,06, N 16,85, S 19,29 %,

gef.: C 57,48, H 5,98, N 16,45, S 18,86 %.

Oxydationen des *l*-Arabinose-1-methylthiobenzhydrazids mit Eisenchlorid bzw. Pyridiniumtribromid ergaben keine Produkte von Interesse.

Eine aus 1,4 g 1-Methylthiobenzhydrazid, 10 ml Alkohol und 5 ml 2-*n*. Salzsäure in der Wärme bereitete Lösung lieferte mit 1,0 g *Benzaldehyd* sogleich eine Emulsion, welche ein viskoses Öl ausschied. Nach dem Impfen mit bei freiwilliger Eindunstung einer Probe des Reaktionsgemisches gewonnenen Kristallen ging das Öl in 1,2 g mit 10 ml gew. Alkohol gewaschene, blassgelbe Nadeln vom Schmp. 118°–125° über, und beim Umkristallisieren dieses Produktes aus 15 ml gew. Alkohol wurden 0,7 g *Benzaldehyd*-1-methylthiobenzhydrazon als goldgelbe, flache Nadeln vom Schmp. 126°–127,5° erhalten.

Für $C_{15}H_{14}N_2S$ (254,34):

ber.: C 70,83, H 5,55, N 11,01, S 12,61 %.

gef.: C 70,85, H 5,60, N 11,08, S 12,54 %.

3. Versuche mit dem 2-Methylthiobenzhydrazid

Nach Eindunstung einer aus 5,7 g *l*-Arabinose-thiobenzhydrazid, 20 ml 1-*n*. Salzsäure und 2,0 g Formalin¹ bereiteten Lösung im Vakuum über Schwefelsäure und Umkristallisierung des Rückstandes aus 20 ml gew. Alkohol wurde die erhaltene Kristallmasse von 4 g Gewicht mit 20 ml konz. Ammoniak versetzt. Hierbei verblieb eine unbedeutende Menge eines weissen Kristallpulvers (5-*l*-Arabinotetraoxybutyl-2-phenyl-1,3,4-thiodiazol) ungelöst, welches am folgenden Tage abfiltriert wurde, wonach das Filtrat bei der freiwilligen Eindunstung freies 2-Methylthiobenzhydrazid als eine klebrige Masse ausschied. Als auch Kristallkrusten von Ammoniumarabonate aufzutreten begannen, wurde das Gemisch mit Wasser versetzt und die ausgeschiedene Base mit 20 ml Äthylazetat aufgenommen. Nach Abfiltrieren von ein wenig auch hier auftretenden Thiodiazols wurde das Filtrat mit geschmolzenem Chlorcalcium getrocknet und dann wurden 5 g *Methyljodid* zugefügt, wonach allmählich 0,7 g eines Additionsproduktes des 2-Methylthiobenzhydrazids mit dem Methyljodid als eine grobkristallinische, blassgelbe Kristallkruste vom Schmp. 123°–125° abgesetzt wurden.

Für $C_9H_{13}N_2JS$ (308,20):

ber.: C 35,07, H 4,25, N 9,09, J 41,18, S 10,41 %.

gef.: C 34,99, H 4,18, N 9,46, J 41,55, S 10,25 %.

Von dem analysierten Produkte ergaben 0,0386 g in Wasser mit Phenolphthalein und 1,25 ml 0,1000-*n*. Natronlauge Rotfärbung und Geruch nach Methylmercaptan. Äquiv.-Gew. ber. 308,2, gef. 309. — Die Mutterlauge des Produktes lieferte bei freiwilliger Eindunstung ein dunkelbraunes, viskoses Öl.

Bei einem neuen Versuche wie oben wurde das 2-Methylthiobenzhydrazid mit Äther aufgenommen, die ätherische Lösung mit 12 ml 1-*n*. Salzsäure ausgeschüttelt und die Säurelösung mit 1 g *Formalin* versetzt. Hierbei trat sofort Emulgierung ein, und ein viskoses Öl wurde ausgeschieden, welches nicht zum Kristallisieren gebracht werden konnte. Es wurde daher mit 20 ml Äther aufgenommen, die ätherische Lösung mit Chlorcalcium getrocknet und mit 6 g Methyljodid versetzt, wonach all-

¹ Vgl. (1, S. 105).

mählich 1,0 g cremefarbene Kristallschuppen ausgeschieden wurden. Bei langsamer Erhitzung schmolz dieses als 4,4-Dimethyl-2-phenyl-1,3,4-thiodiazoliniumjodid (X) formulierte Produkt bei ca. 184°–186°, bei schneller Erhitzung bei 192°–193°.

Für $C_{10}H_{13}N_3JS$ (320,21):

ber.: C 37,51, H 4,09, N 8,75, J 39,64, S 10,01 %,

gef.: C 37,39, H 4,10, N 8,73, J 39,78, S 10,07 %.

Wie unten dargelegt wird, entsteht dieselbe Verbindung auch bei Umsetzungen des Methyljodids mit Methylenthio benzhydraziden.

Als eine wie oben bereitete, salzsaure Lösung des 2-Methylthio benzhydrazids mit einer Lösung von 1,5 g *l*-Arabinose in 10 ml Wasser versetzt wurde, entstand ein homogenes Gemisch, welches bald farblose, flache Prismen abzuscheiden begann. Das am folgenden Tage abgesaugte und mit 10 ml Wasser gewaschene Produkt wog 1,2 g und schmolz bei 142°–148° zu einer braunen Schmelze (Präp. 1). Beim Umkristallisieren von 2,0 g solcher Produkte aus 20 ml gew. Alkohol wurden 1,45 g dichte Aggregate von kleinen Nadelchen vom Schmp. 140°–146° erhalten (Präp. 2).

Für $C_{13}H_{18}O_4N_2S$ (298,36):

ber.: C 52,33, H 6,08, N 9,39, S 10,75 %,

für Präp. 1 gef.: C 52,23, H 6,01, N 9,35, S 10,83 %,

für Präp. 2 gef.: C 52,35, H 6,08, N 9,35, S 10,70 %.

Eine Lösung von 0,4044 g des Präp. 1 in Pyridin auf 10,00 ml Volumen gelöst zeigte t Minuten nach dem Zusatz des Lösungsmittels:

t :	5	10	15	25	50	100
α_D^{20} :	$-2,17^\circ$	$-4,05^\circ$	$-4,24^\circ$	$-4,30^\circ$	$-4,30^\circ$	$-4,30^\circ$
$[\alpha]_D^{20} =$	$\xrightarrow{t=5}$	$-53,7^\circ$	$\xrightarrow{t=25}$	$-106,3^\circ$		

In der gleichen Weise zeigten 0,4073 g des Präp. 2:

t :	6	10	20	40	
α_D^{20} :	$-4,57^\circ$	$-4,30^\circ$	$-4,28^\circ$	$-4,28^\circ$	
$[\alpha]_D^{20} =$	$\xrightarrow{t=6}$	$-112,2^\circ$	$\xrightarrow{t=20}$	$-105,1^\circ$	$[M]_D^{20} = \xrightarrow{t=20} -313,6^\circ$

Nach einstündigem Schütteln von 3,0 g des Präp. 1 mit 25 ml 0,5-*n*. Natronlauge waren 2,0 g Pulver vom Schmp. 142°–148° und von $[\alpha]_D^{20} = \rightarrow -103,0^\circ$ (Pyridin) ungelöst verblieben; das Filtrat davon lieferte mit 10 ml 2-*n*. Essigsäure eine Emulsion, welche eine unbedeutende Menge von klebrigen Kristallwarzen ausschied. — Nach der gleichen Behandlung von 2,6 g desselben Präparates mit 20 ml 1-*n*. Salzsäure waren 2,0 g vom Schmp. 142°–149° ungelöst verblieben. — Eine Lösung von 2,3 g desselben Produktes in 20 ml heissem, 50 %igem Alkohol hielt sich beim Erkalten unverändert, und nach dem Zusatz von 2 g Formalin schied sie bis zum folgenden Tage 0,9 g unverändertes Ausgangsmaterial vom Schmp. 143°–147° und (in Pyridin) von $[\alpha]_D^{20} = \rightarrow -106,2^\circ$ aus, während das Filtrat davon bei der freiwilligen Eindunstung nichts von Interesse lieferte.

Als eine Lösung von 1,0 g chlorwasserstoffsäurem 2-Methylthio benzhydrazid in 10 ml Wasser mit der gleichen Menge Benzaldehyd in 10 ml Alkohol versetzt wurde,

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trat sofort Emulgierung ein und ein Öl wurde ausgeschieden, welches bis zum folgenden Tage in 0,8 g eines farblosen Kristallkuchens vom Schmp. 63°–65° überging (XIa).

Für $C_{15}H_{14}N_2S$ (254,34):

ber.: C 70,83, H 5,55, N 11,01, S 12,61 %,

gef.: C 70,76, H 5,51, N 10,98, S 12,63 %.

Als bei der Schmelzpunktbestimmung das Erhitzen während 10 Minuten bei ca. 80° fortgesetzt wurde, erstarrte die Schmelze, wonach die Probe aufs Neue bei 156°–157° wieder flüssig wurde. In zugeschmolzenem Röhrchen schmolz das Produkt bei 64°–65° und war dann nach 10 Minuten bei ca. 90° immer noch flüssig; nach beim Erkalten eingetretener Erstarrung trat wie vorher Schmelzen bei 64°–65° ein. — Beim Erhitzen von 0,55 g des Produktes 10 Minuten auf dem Wasserbade nahm das Gewicht mit 0,02 g zu, und beim Erkalten wurde eine Kristallmasse erhalten, welche den Schmp. ca. 145°–150° aufwies. Nach Pulverisierung wurde die Probe noch 20 Minuten wie vorher erhitzt, wonach sie bei ca. 145°–152° schmolz (Präp. 1). — Beim Umkristallisieren von 2 g wie oben dargestellten 4-Methyl-2,5-diphenyl-1,3,4-thiodiazolins aus 20 ml abs. Alkohol wurden 1,6 g schmale, gelbliche Prismen vom Schmp. ca. 140°–155° erhalten, und nach noch einer Umkristallisierung aus 40 ml Alkohol zeigte das Produkt den Schmp. 157°–158° (Präp. 2). — Beim Schütteln von 1,0 g desselben Thiodiazolins mit 25 ml gew. Alkohol trat fast vollständiges Auflösen ein, und beim Versetzen der Lösung mit einer Lösung von 1,5 g Pyridiniumtribromid vom J-Äquiv.-Gew. 174 in 10 ml Alkohol trat eine schwache Wärmeentwicklung ein und das Gemisch entfärbte sich allmählich. Bei freiwilliger Eindunstung des Reaktionsgemisches wurde nur eine unbedeutende Menge Kristalle abgeschieden; als 13 ml 1-n. Natronlauge zugefügt wurden, trat Emulgierung ein, wonach 0,6 g cremefarbene, schmale Täfelchen vom Schmp. 152°–154° ausgeschieden wurden (Präp. 3). Bei den Analysen zeigten sämtliche drei Präparate die Zusammensetzung des 4-Methyl-2,5-diphenyl-5-oxy-1,3,4-thiodiazolins (XIb):

Für $C_{15}H_{13}ON_2S$ (270,34):

ber.: C 66,64, H 5,22, N 10,36, S 11,86 %,

für Präp. 1 gef.: C 66,37, H 5,22, N 9,99, S 11,86 %,

für Präp. 2 gef.: C 66,47, H 5,22, N 10,30, S 11,74 %,

für Präp. 3 gef.: C 66,11, H 5,20, N 10,70, S 11,42 %.

4. Versuche mit dem Thiobenzhydrazid

Aus 3 g Thiobenzhydrazid in 20 ml 1-n. Natronlauge und 4 g *Methyljodid* in 20 ml Alkohol wurde beim Schütteln unter schwacher Wärmeentwicklung ein homogenes Gemisch erhalten, welches sich bald zu trüben begann und bis zum folgenden Tage 1,2 g gelbe Prismen vom Schmp. ca. 173°–177° ausschied. Beim Umkristallisieren aus 50 ml gew. Alkohol lieferte das Produkt 1,0 g reines 3,6-Diphenyl-1,4-dihydro-1,2,4,5-tetrazin vom Schmp. 191°–193°.

Für $C_{14}H_{12}N_4$ (236,27):

ber.: C 71,16, H 5,12, N 23,72 %,

gef.: C 71,04, H 5,09, N 23,78 %.

Das Filtrat von dem Rohprodukte lieferte bei der weiteren Bearbeitung nichts von Interesse.

Als eine Lösung von 6,1 g Thiobenzhydrazid in 12 ml Äthylazetat mit 6 g Methyljodid versetzt wurde, entstand ein klares Gemisch, welches sich kaum merkbar erwärmte und sich nach ca. 20 Minuten zu trüben begann, wonach es nach ein Paar Tagen 6,9 g schwach cremefarbenes Pulver vom Schmp. ca. 112° – 120° abgesetzt hatte. Bei der Behandlung dieses Pulvers mit insgesamt 100 ml Wasser verblieben 2,7 g Kristallpulver vom Schmp. 135° – 138° ungelöst; bei der Umkristallisierung aus Benzol lieferte dieses Produkt reines 2,5-Diphenyl-1,3,4-thiodiazol als farblose, flache Prismen vom Schmp. 141° – 142° .

Für $C_{14}H_{10}N_2S$ (238,30):

ber.: C 70,56, H 4,23, N 11,76, S 13,45 %.

gef.: C 70,29, H 4,27, N 11,81, S 13,34 %.

Das Benzolfiltrat roch nach Methylmercaptan bzw. Dimethylsulfid und lieferte bei freiwilliger Eindunstung eine rotbraune, stark ölige Kristallmasse, welche nichts von Interesse ergab. — Die aus dem Rohprodukt oben mit Wasser erhaltene Lösung lieferte bei freiwilliger Eindunstung eine feuchte Kristallmasse, welche nach dem Trocknen auf Tonscherben 1,6 g wog und bei 124° – 126° schmolz. Bei der Behandlung dieser Masse mit 5 ml Wasser verblieb ein wenig eines bräunen, klebrigen Öles ungelöst, und nach dem Abfiltrieren davon und Eindunstung des Filtrates fast zur Trockene wurden auf Tonscherben 1,1 g Hydraziniumjodid vom Schmp. 126° – 128° (unter heftiger Gasentwicklung)¹ erhalten.

Für H_5N_2J (159,98):

ber.: H 3,15, N 17,51, J 79,33 %,

gef.: H 3,16, N 17,60, J 79,17 %.

Bei einem gleichartigen Versuche wurde zum Schluss eine Salzmasse erhalten, welche bei 115° – 117° schmolz und gemäss der Analyse aus einem Gemisch von Hydraziniumjodid mit etwas Trimethylsulfoniumjodid bestand.

Für $0,833 H_5N_2J + 0,167 C_3H_9JS$ (167,35):

ber.: C 3,60, H 3,41, N 13,95, J 75,84, S 3,20 %,

gef.: C 3,62, H 3,40, N 14,07, J 76,08, S 3,10 %.

Bei einem dritten Versuche, aber mit 3,1 g Thiobenzhydrazid in 20 ml Äthylazetat und 6 g Methyljodid, wurden nach 3 Tagen 2,4 g eines Reaktionsproduktes erhalten, welches bei der Umkristallisierung aus gew. Alkohol 2,5-Diphenyl-1,3,4-thiodiazol vom Schmp. 140° – 142° lieferte, während die Mutterlauge nach dem Zusatz von Benzaldehyd bald Benzalazin als gelbe, nadelförmige Prismen vom Schmp. 92° – 93° abzuscheiden begann.

Für $C_{14}H_{12}N_2$ (208,25):

ber.: C 80,74, H 5,81, N 13,45 %,

gef.: C 80,38, H 6,05, N 13,91 %.

¹ CURTIUS und SCHULZ fanden (13) für dieses Salz den Schmp. 127° (unter heftiger Verpuffung).

Auch bei der Umsetzung des Benzaldehyd-thiobenzhydrazons mit Methyljodid in Äthylazetat entstand das 2,5-Diphenyl-1,3,4-thiodiazol, und diese Verbindung wurde auch erhalten, als das Thiobenzhydrazid in demselben Lösungsmittel mit Monojessigsäure umgesetzt wurde.

5. Versuche mit Methylthiobenzhydraziden

A. Versuche mit dem Methylthiobenzhydrazid

Als 2,0 g in früher (2) beschriebener Weise dargestelltes, chlorwasserstoffsäures *Methylthiobenzhydrazid* mit 20 ml Äther und 11 ml 1-*n*. Sodalösung geschüttelt wurden, entstanden unter Entwicklung von Kohlendioxyd homogene Flüssigkeitsschichten, von denen die ätherische nach Trocknen mit geschmolzenem Chlorcalcium beim Vertreiben des Äthers vor der Wasserstrahlpumpe 1,3 g eines auf Tonscherben getrockneten Rückstandes vom Schmp. ca. 40° lieferte. Dieses Produkt war indessen weder in 1-*n*. Natronlauge oder in 2-*n*. Salzsäure löslich, und bei der Fällung seiner Lösung in Benzol mit Petroläther wurde nur unreines Methylen-bis-methylthiobenzhydrazid vom Schmp. ca. 75°–88° erhalten. — Bei einem ähnlichen Versuche, aber mit Benzol als Extraktionsmittel, wurde nach freiwilligem Verdunsten des Benzols und Trocknen des Rückstandes auf Tonscherben 1,4 g einer Kristallmasse erhalten, welche bei der Behandlung mit abs. Alkohol 0,7 g des genannten bis-Derivates als farblose Nadelchen vom Schmp. 89°–90° lieferte.

Für $C_{17}H_{16}N_4S_2$ (340,46):

ber.: C 59,97, H 4,74, N 16,46, S 18,83 %,

gef.: C 59,82, H 4,69, N 16,41, S 18,41 %.

Wie an anderer Stelle (9) mitgeteilt wird, geht das Methylthiobenzhydrazid beim Oxydieren mit Eisenchlorid oder Pyridiniumtribromid in das 2-Phenyl-1,3,4-thiodiazol über; als danach 2,5 g dieser Verbindung mit 2,5 g *Methyljodid* versetzt wurden, entstand eine homogene Flüssigkeit, welche bis zum folgenden Tage in einen Kristallbrei überging. Auf Tonscherben wurden hieraus 2,1 g eines Rückstandes erhalten, welcher bei ca. 130°–175° schmolz und beim Umkristallisieren aus 10 ml gew. Alkohol 1,1 g schwach gelbliche, farnartig vereinigte, flache Prismen vom Schmp. 181°–183° (Schmelze blasig) lieferte. Die Analyse ergab die Zusammensetzung eines Methylphenylthiodiazoliumjodids (XV):¹

Für $C_9H_9N_2JS$ (304,16):

ber.: C 35,54, H 2,98, N 9,21, J 41,73, S 10,54 %,

gef.: C 35,45, H 3,02, N 9,26, J 41,65, S 10,56 %.

Von dem analysierten Produkte wurden 0,3306 g in 10 ml Wasser gelöst, mit Phenolphthalein versetzt und mit 0,1000-*n*. Natronlauge titriert, wobei nach vorübergehender Gelbfärbung ein etwas unscharfer Umschlag mit 21,60 ml der Lauge erhalten wurde, Äquiv.-Gew. ber. 152,1, gef. 153,1. Nach Zusatz von 12,20 ml der Lauge wurde die Lösung dann unscharf von 10,30 ml 0,1000-*n*. Salzsäure entfärbt.

¹ Nach 3 Monaten bei gewöhnlicher Temperatur war in einem Gemisch aus 2,4 g 2,5-Diphenyl-1,3,4-thiodiazol, 25 ml Äthylazetat und 5 g Methyljodid keine merkbare Umsetzung eingetreten.

Als 2,3 g des Jodids mit 0,5 g Natriumbisulfit in 10 ml Wasser und 20 ml 1-n. Natronlauge versetzt wurden, entstand eine Emulsion, welche eine unbedeutende Menge einer teerigen Masse absetzte. Die davon abdekantierte Lösung wurde von 5 ml 2-n. Essigsäure emulgiert und schied dann 1,3 g des dem Jodide entsprechenden Hydroxyds als grüngelbliche, dicke Prismen vom Schmp. 75°–77° aus.

Für $C_9H_{10}ON_2S$ (194,25):

ber.: C 55,64, H 5,19, N 14,42, S 16,51 %.

gef.: C 55,54, H 5,20, N 14,46, S 16,51 %.

In Übereinstimmung mit dem Ergebnisse der alkalimetrischen Titrierung oben war das erhaltene Hydroxyd sowohl in Natronlauge als auch in Salzsäure löslich, und beim Titrieren von 0,1994 g davon in 10 ml Alkohol wurde mit Phenolphthalein und 10,20 ml 0,1000-n. Natronlauge ein ziemlich guter Umschlag erhalten, Äquiv.-Gew. ber. 194,3, gef. 195,5. — Als 0,8 g des Hydroxyds mit 10 ml Äther und 8 ml 2-n. Salzsäure geschüttelt wurden, entstand eine farblose Wasserlösung, welche bei freiwilliger Eindunstung das entsprechende Chlorid zurückliess. Nach dem Trocknen auf Tonscherben wog dieses Salz 0,5 g und schmolz unter Gasentwicklung bei 169°–170°.

Für $C_9H_9N_2ClS$ (212,70):

ber.: Cl 16,67 %; gef.: Cl 16,67 %.

Von dem Salz ergaben 0,2037 g in 10 ml Wasser mit Phenolphthalein und 18,40 ml 0,1000-n. Natronlauge einen ziemlich guten Umschlag, Äquiv.-Gew. ber. 106,3, gef. 110,7, wonach die Lösung nach Zusatz von Methylrot von 8,5 ml 0,1-n. Salzsäure unscharf rotgelb wurde.

Beim Schütteln von 4,0 g chlorwasserstoffsäurem Methylenthio benzhydrazid mit 25 ml Benzol und 21 ml 1-n. Sodalösung entstand eine schwach milchige Wasserlösung und eine klare, gelbe Benzolschicht. Diese wurde mit geschmolzenem Chlormalcium getrocknet und dann mit 5 g *Methyljodid* versetzt, wonach das Gemisch sich nach etwa einer Stunde zu trüben begann und dann allmählich 3 g gelbes Kristallpulver vom Schmp. ca. 160°–165° absetzte. Bei der Umkristallisierung aus 50 ml Wasser lieferte dieses Pulver 1,2 g schwach cremefarbene Schuppen vom Schmp. 192°–194° (bei schneller Erhitzung); denselben Schmp. zeigte ein Gemisch aus diesem Produkt und dem entsprechenden S. 11.

Für $C_{10}H_{13}N_2JS$ (320,21):

ber.: C 37,51, H 4,09, N 8,75, J 39,64, S 10,01 %.

gef.: C 37,34, H 4,04, N 8,73, J 39,49, S 10,11 %.

Gegen Phenolphthalein verbrauchten 0,3588 g dieses Produktes in 20 ml warmem Wasser zuerst und mit gleitendem Umschlag 2,50 und dann bei fortgesetzter Erwärmung insgesamt noch 9,00 ml 0,1000-n. Natronlauge, Äquiv.-Gew. ber. 320, gef. 312.

Nach Schütteln von 4,0 g Methylenthio benzhydrazid-hydrochlorid mit 40 ml 1-n. Natronlauge und Abfiltrieren einer geringen Menge einer ungelösten, zusammengeballten Masse wurde das milchige Filtrat mit 3,5 g mit 25 ml 1-n. Natronlauge neutralisierter *Monobromessigsäure* versetzt, wonach bis zum folgenden Tage 2,1 g Methylen-bis-methylen-thio benzhydrazid vom Schmp. ca. 85°–90°, nach Umkristal-

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lisieren aus gew. Alkohol 89°–90°, ausgeschieden wurden. Das Filtrat davon wurde von 15 ml 5-n. Salzsäure emulgiert und schied danach eine Kristallkruste aus, welche beim Umkristallisieren aus 20 ml gew. Alkohol 0,3 g Nadeln vom Schmp. 183°–184° lieferte. Die Analyse dieser Verbindung ergab die Zusammensetzung des 4,4'-Methylen-bis-5,6-dihydro-2-phenyl-5-oxo-1,3,4-thiodiazins (XVI):

Für $C_{19}H_{10}O_2N_4S_2$ (396,48):

ber.: C 57,55, H 4,07, N 14,13, S 16,18 %,

gef.: C 57,76, H 4,08, N 14,17, S 16,20 %.

Als schliesslich 5,7 g desselben Hydrochlorids mit 6 g *Thioglykolsäure* in 6 ml Wasser geschüttelt wurden, entstand bald ein homogenes Gemisch, welches allmählich eine weiche Kristallmasse ausschied. Nach Zusatz von mehr Wasser und Kühlung im Eisschrank wurden hiervon 4,1 g eines Produktes erhalten, welches nach zweimaliger Umkristallisation aus gew. Alkohol und dann aus Benzol aus schwach cremefarbenen Schuppen oder dünnen Tafelchen vom Schmp. 103°–104° und der Zusammensetzung des 3-Thiobenzoylamino-4-oxo-1,3-thiazolidins (XVII) bestanden.

Für $C_{10}H_{10}ON_2S_2$ (238,33):

ber.: C 50,39, H 4,24, N 11,76, S 26,91 %,

gef.: C 50,70, H 4,24, N 11,86, S 26,70 %.

B. Versuche mit dem Methylen-bis-methylthiobenzhydrazid

Als 2,3 g Methylen-bis-methylthiobenzhydrazid (2) mit einer Lösung von 5,2 g *Pyridiniumtribromid* vom J-Äquiv.-Gew. 179 in 25 ml gew. Alkohol behandelt wurden, entstand schnell und unter schwacher Wärmeentwicklung eine schwach gelbrote Lösung, welche am folgenden Tage mit 25 ml Wasser versetzt wurde, wonach bei freiwilliger Eindunstung des Gemisches 1,5 g des in der gleichen Weise (9) aus dem Methylthiobenzhydrazid gewonnenen 2-Phenyl-1,3,4-thiodiazols vom Schmp. 39°–41° ausgeschieden wurden.

Für $C_8H_6N_2S$ (162,21):

ber.: N 17,27, S 19,77 %; gef.: N 17,32, S 19,64 %.

Beim Versetzen von 3,2 g Methylen-bis-methylthiobenzhydrazid in 25 ml Äthylazetat mit 5 g *Methyljodid* entstand ein homogenes Gemisch, welches bis zum folgenden Tage 3,1 g schwach gelbliche, flache Prismen vom Schmp. ca. 180°–182° (Zersetzung) ausschied. Beim Umkristallisieren aus 50 ml Wasser lieferten 1,8 g davon 1,3 g fast weisse Schuppen oder dünne Tafelchen vom Schmp. 192°–193° (bei schnellem Erhitzen und unter Zersetzung) und auch von der Zusammensetzung des in der gleichen Weise oben aus dem Methylthiobenzhydrazid gewonnenen Produktes (X).

Für $C_{10}H_{13}N_2JS$ (320,21):

ber.: C 37,51, H 4,09, N 8,75, J 39,64, S 10,01 %,

gef.: C 37,38, H 4,05, N 8,78, J 39,75, S 10,05 %.

Aus 6,8 g Methylen-bis-methylenthio benzhydrazid und 10 g Thioglykolsäure entstand ein homogenes Gemisch, welches nach einer Woche nur etwas viskoser geworden war. Bei der Behandlung des Gemisches mit 23,5 g Soda in 100 ml Wasser verblieben dann 4,4 g einer teigigen Masse ungelöst, während die entstandene Lösung beim Ansäuern mit Salzsäure nur klebrige, nicht kristallisierbare Produkte lieferte. Die von der Sodalösung ungelöste Masse ergab beim Umkristallisieren aus 20 ml Benzol 1,2 g schwach cremefarbene, dünne Täfelchen vom Schmp. 103° – 104° und auch von der Zusammensetzung des oben als 3-Thiobenzoylamino-4-oxo-1,3-thiazolidin (XVII) aufgefassten Produktes.

Für $C_{10}H_{10}ON_2S_2$ (238,33):

ber.: C 50,39, H 4,24, N 11,76, S 26,91 %.

gef.: C 50,56, H 4,20, N 11,76, S 26,70 %.

Aus einem homogenen Gemisch aus 1,9 g Methylen-bis-methylenthio benzhydrazid und 4 g Thioglykolsäure entstand beim Zusatz von 6 ml 2-n. Salzsäure eine Emulsion, welche bis zum folgenden Tage ein viskoses Öl ausschied. Dieses Öl wurde mit Äther aufgenommen, der Äther abdestilliert und der Rückstand vor der Wasserstrahlpumpe auf dem Wasserbade erhitzt, wobei 2,6 g einer braunen, sirupösen Masse erhalten wurden. Diese Masse verbrauchte in ätherischer Lösung und gegen Phenolphthalein 9 ml 1-n. Natronlauge, wonach die Ätherlösung bei freiwilliger Eindunstung 1,2 g eines kristallinischen Produktes lieferte. Beim Umkristallisieren aus 40 ml gew. Alkohol lieferte dieses Produkt 0,8 g gelbliche Nadeln vom Schmp. 103° – 105° und von der Zusammensetzung eines durch Wasserabspaltung ringgeschlossenen Additionsproduktes des Methylen-bis-methylenthio benzhydrazids mit der Thioglykolsäure (XVIII).

Für $C_{10}H_{10}ON_2S_3$ (414,56):

ber.: C 55,04, H 4,38, N 13,52, S 23,20 %.

gef.: C 55,18, H 4,42, N 13,45, S 23,20 %.

C. Versuche mit dem Methylen-oxymethylthio benzhydrazid

Das Methylen-oxymethylthio benzhydrazid, welches zuerst bei der Behandlung des *l*-Arabinose-thio benzhydrazids mit Formaldehyd in alkalischer Lösung erhalten wurde (1, S. 97), lässt sich auch direkt aus dem Thiobenzhydrazid selber und Formaldehyd in alkalischem Gemische darstellen. Bei einem Versuche hierüber wurde eine Lösung von 3,1 g Thiobenzhydrazid in 20 ml 1-n. Natronlauge mit 4 g mit 16 ml Wasser verdünntem Formalin versetzt, wonach das homogene Gemisch sich schnell emulgierte und nach 2 Stunden 3,5 g mit 10 ml Wasser gewaschene und zwischen Filtrierpapier abgepresste, schmale, farblose, glänzende Schuppen vom Schmp. 62° – 64° ausgeschieden hatte.

Für $C_9H_{10}ON_2S$ (194,25):

ber.: C 55,64, H 5,19, N 14,42, S 16,51 %.

gef.: C 55,35, H 5,16, N 14,40, S 16,44 %.

Beim Verwahren an der Luft verloren 0,6118 g des erhaltenen Produktes im Laufe von zwei Monaten 0,0772 g oder 12,62 % am Gewicht, während die Gleichung



einen Gewichtsverlust von 12,36 % erfordert. Der Rückstand bestand dann aus einer gelblichen, etwas zusammengesinterten Kristallmasse vom Schmp. ca. 83°–86° und von der Zusammensetzung des Methylen-bis-methylthiobenzhydrazids.

Für $C_{17}H_{16}N_4S_2$ (340,46):

ber.: C 59,97, H 4,74, N 16,46, S 18,83 %,

gef.: C 60,16, H 4,67, N 16,56, S 18,42 %.

Dass das Methylen-oxymethylthiobenzhydrazid in saurer Lösung schnell derselben Zersetzung unterliegt, wurde schon früher (a. a. O.) erwähnt. Auch in eigener und in alkalischer Lösung spielt sich derselbe Vorgang ab, denn eine Lösung von 3,3 g der Verbindung in 20 ml gew. Alkohol schied im Laufe von 3 Tagen 1,7 g farblose Nadeln vom Schmp. 88°–90° aus, und eine Lösung von 1,0 g davon in 10 ml mit 1,0 ml 1-n. Natronlauge versetztem Alkohol setzte bis zum folgenden Tage 0,1 g und dann während 3 Tagen noch 0,15 g Nadeln vom Schmp. 89°–90° ab.

Als eine Lösung von 3,2 g Methylen-oxymethylthiobenzhydrazid in 20 ml Äthylazetat mit 5 g *Methyljodid* versetzt wurde, entstand ein homogenes Gemisch, welches am folgenden Tage eine kleine Menge feiner Nadeln ausgeschieden hatte, und nach 13 Tagen wurden beim Absaugen, Waschen mit 10 ml Äthylazetat und Trocknen an der Luft 3,4 g gelbliche Nadeln vom Schmp. 187°–189° (Zersetzung) erhalten. Beim Umkristallisieren dieses Produktes aus einem Gemische von 50 ml Alkohol mit 25 ml Wasser wurden 2,2 g mit 10 ml Alkohol gewaschene, glänzende Schuppen oder dünne Täfelchen erhalten, welche bei 193°–194° (bei schneller Erhitzung und unter Zersetzung) schmolzen und auch die gleiche Zusammensetzung wie die aus den obigen Methylthiobenzhydraziden erhaltenen Methyljodid-Produkte (X) aufwiesen.

Für $C_{16}H_{13}N_2JS$ (320,21):

ber.: C 37,51, H 4,09, N 8,75, J 39,64, S 10,01 %,

gef.: C 37,78, H 4,10, N 8,30, J 39,87, S 10,28 %.

Die Mutterlauge des Rohproduktes lieferte bei freiwilliger Eindunstung bis zu 0,9 g Gewicht eine braungelbe Flüssigkeit, welche mit Wasser mischbar war und stark nach Formaldehyd roch.

Akarp, Schweden, Mai 1955.

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Tryckt den 6 december 1955

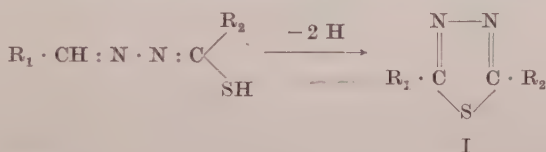
Uppsala 1955. Almqvist & Wiksells Boktryckeri AB

Oxydationsprodukte einiger Thiobenzhydrazone und Thiosemicarbazone

VON BROR HOLMBERG

1. Einleitung und Übersicht

Bei Behandlungen des Thiobenzhydrazids mit schwachen Oxydationsmitteln erhielt der Verf. das 2,5-Diphenyl-1,3,4-thiodiazol (I, $R_1 = R_2 = C_6H_5$), und diese Verbindung entstand auch, als das Thiobenzhydrazon des Benzaldehyds mit Eisenchlorid oxydiert wurde (1). In diesem Falle war indessen die Ausbeute an dem Thiodiazol so hoch, dass auch eine Reaktion analog der von YOUNG und EYRE (2) bei dem Thiosemicarbazon des Benzaldehyds gefundenen ($R_1 = C_6H_5$, $R_2 = NH_2$):



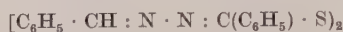
mitgewirkt haben dürfte.

Gemäss dem angeführten Schema lieferte das Glykolaldehyd-thiobenzhydrazon das 5-Oxymethyl-2-phenyl-1,3,4-thiodiazol (I, $R_1 = HOCH_2$, $R_2 = C_6H_5$), und aus einer Anzahl Aldose-thiobenzhydrazonen (bzw. -thiobenzhydraziden) wurden 5-Polyoxyalkyl-2-phenyl-1,3,4-thiodiazole (I, $R_1 = C_nH_{2n+1}O_n$) gewonnen. Solche Produkte wurden auch bei den Oxydationen von Ketose-thiobenzhydrazonen erhalten (3), wobei also eine Sprengung des Kohlenstoffskelettes eingetreten sein musste, und es erschien daher von einigem Interesse, zu untersuchen, inwieweit andere Keto-thiobenzhydrazone in der gleichen Weise zersetzt werden können, und ob auch Keto-thiosemicarbazone einer ähnlichen Umsetzung fähig sind.

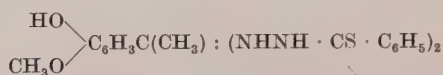
Bei den zu dem genannten Zwecke ausgeführten Versuchen wurde aus dem Thiobenzhydrazon der *Brenztraubensäure* das in der gleichen Weise aus dem Thiobenzhydrazon des *Azetalddehyds* erhältliche 5-Methyl-2-phenyl-1,3,4-thiodiazol (I, $R_1 = CH_3$, $R_2 = C_6H_5$) erhalten, während die Thiobenzhydrazone des *Azetons*, des *Azetessigesters* und des *Azetoveratrons* bei den Oxydationen höchstens kleine Mengen des aus dem Thiobenzhydrazidreste allein entstehenden 2,5-Diphenyl-1,3,4-thiodiazol nebst undefinierbaren, klebrigen Produkten lieferten.

Das früher (3, S. 81 und 82) als Oxydationsprodukt des Glykolaldehydthiobenzhydrazons und der entsprechenden Verbindungen der *d*-Fructose und der *l*-Sorbose erhaltene 5-Oxymethyl-2-phenyl-1,3,4-thiodiazol wurde jetzt in der gleichen Weise aus dem Thiobenzhydrazon des *Dioxyazetons* gewonnen. — Die Überführung des

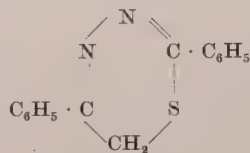
Benzaldehyd-thiobenzhydrazons in 2,5-Diphenyl-1,3,4-thiodiazol gelang jetzt auch mit Wasserstoffsuperoxyd als Oxydationsmittel, während Kaliumpersulfat es in das in zwei Formen entstehende Disulfid seiner *iso*-Form (II) überführte. — Die Darstellung eines Thiobenzhydrazons des *Benzoylcarbinols* gelang nicht, und bei einem Versuche, das *Phenazylbromid* mit dem Thiobenzhydrazid umzusetzen, trat auch Abspaltung von Bromwasserstoff ein, wobei das 2,5-Diphenyl-1,3,4-thiodiazin (III) entstand.¹ Dagegen lieferte das *Phenazylazetat* ein Thiobenzhydrazon, welches indessen keine definierbaren Oxydationsprodukte lieferte.



II



IV



III

Aus *Benzil* und Thiobenzhydrazid entstand ein Mono-thiohydrazon, welches bei der Oxydation mit Eisenchlorid mit ziemlich guter Ausbeute 2,5-Diphenyl-1,3,4-thiodiazol nebst kleinen Mengen von Benzoesäure lieferte. — *Vanillin* setzte sich mit dem Thiobenzhydrazid zu einem Hydrazon um, welches bei der Behandlung mit Eisenchlorid das zu erwartende 2-Phenyl-5-guajacyl-1,3,4-thiodiazol (I, $\text{R}_1 =$

$\begin{array}{c} \text{HO} \\ \text{CH}_3\text{O} \end{array} \text{C}_6\text{H}_3, \text{R}_2 = \text{C}_6\text{H}_5$) ergab. Aus dem *Azetovanillon* und Thiobenzhydrazid

wurde kein Thiohydrazon sondern ein *bis*-Thiobenzhydrazid (IV) gebildet. — Sowohl der *Veratrumaldehyd* wie das *Azetoveratron* konnten zu Thiobenzhydrazonen übergeführt werden, von denen das Aldehydderivat beim Oxydieren 2-Phenyl-5-dimethoxyphenyl-1,3,4-thiodiazol (I, $\text{R}_1 = (\text{CH}_3\text{O})_2\text{C}_6\text{H}_3, \text{R}_2 = \text{C}_6\text{H}_5$), das Ketonderivat aber nur teigige Massen lieferten.

Eine oxydative Sprengung der Kohlenstoffkette ist also nur bei Keton-thiobenzhydrazonen eingetreten, welche eine Carbinol-, Keto- oder Carboxylgruppe an Carbonylkohlenstoff gebunden enthalten. Bei den obenerwähnten Thiobenzhydrazonen von α -Oxyaldehyden wurde zwar keine ähnliche Reaktion beobachtet, aber als jetzt das Thiobenzhydrazon der *Glyoxylsäure* mit Eisenchlorid behandelt wurde, trat Abspaltung von Kohlendioxyd ein und das 2-Phenyl-1,3,4-thiodiazol (I, $\text{R}_1 = \text{H}, \text{R}_2 = \text{C}_6\text{H}_5$) entstand.

Die Thiosemicarbazone verhielten sich dem Eisenchlorid gegenüber analog den Thiobenzhydrazonen. Hierbei wurde aus den Thiosemicarbazonen des *Azetaldehyds*, des *Oxyazetons* und des *Methyl- α -oxyäthylketons* das Hydrochlorid des 5-Methyl-2-amino-1,3,4-thiodiazols (I, $\text{R}_1 = \text{CH}_3, \text{R}_2 = \text{NH}_2$) erhalten, während das Thiosemicarbazon des *Azetons* nur teigige, nicht kristallisierbare Produkte lieferte. — Aus dem Thiosemicarbazon der *l-Arabinose* wurde das Hydrochlorid des 5-*l*-Arabinotetraoxybutyl-2-amino-1,3,4-thiodiazols (I, $\text{R}_1 = \text{C}_4\text{H}_9\text{O}_4, \text{R}_2 = \text{NH}_2$) und aus dem entsprechenden Derivat der *d-Fructose* die *d*-Form desselben Salzes erhalten. — Das Thiosemicarbazon des *Cyclohexanol-1-on-2* ergab keine definierbaren Produkte mit Eisenchlorid, während aus den Thiosemicarbazonen des *Benzaldehyds*, des *Benzoyl-*

¹ Gemäss BOSE (4) und SANDSTRÖM (5) können solche Verbindungen auch aus dem Phenazylbromid und Dithiocarbazinaten dargestellt werden.

carbinols, des Benzoin's und der Phenylglyoxylsäure das Hydrochlorid des 5-Phenyl-2-amino-1,3,4-thiodiazols (I, $R_1 = C_6H_5$, $R_2 = NH_2$) gebildet wurde. — Aus dem Kondensationsprodukte des Phenylglyoxals mit dem Thiosemicarbazid entstand bei der Behandlung mit Eisenchlorid das Hydrochlorid des 5-Benzoyl-2-amino-1,3,4-thiodiazols (I, $R_1 = C_6H_5CO$, $R_2 = NH_2$), woraus hervorgeht, dass die Aldehydgruppe des Phenylglyoxals sich mit dem Thiosemicarbazid kondensiert hatte. — Behandlungen der Thiosemicarbazone des Propiophenons und des Phenylazetons mit Eisenchlorid ergaben keine kristallisierbaren Produkte.

Diese Untersuchung wurde in der damaligen org.-chemischen Abteilung des Nobelinstitutes der Kgl. Akademie der Wissenschaften, Stockholm, begonnen und in der entsprechenden Abteilung des Universitätslaboratoriums in Lund abgeschlossen. — Die Elementaranalysen wurden von Frau MAJBRIIT VESTERGAARD in dem genannten Nobelinstitute ausgeführt.

2. Oxydationsprodukte einiger Thiobenzhydrazone

A. 2-Phenyl-1,3,4-thiodiazol

Das 2-Phenyl-1,3,4-thiodiazol wurde zuerst durch Entcarboxylierung der als Oxydationsprodukt des 5-*l*-Arabinotetraoxybutyl-2-phenyl-1,3,4-thiodiazols erhaltenen Phenylthiodiazolcarbonsäure (3, S. 73) gewonnen. In kleiner Menge entstand es auch beim Oxydieren des *l*-Arabinose-methylthiobenzhydrazids mit Eisenchlorid (3, S. 108), und jetzt ist es auch bei Oxydationen des Methylthiobenzhydrazids und des Glyoxylsäure-thiobenzhydrazons sowie durch Erhitzung des Thiobenzhydrazids mit Ameisensäure erhalten worden.¹

Bei den Versuchen mit dem Methylthiobenzhydrazid wurden zuerst 2 g des wie früher (1) dargestellten Hydrochlorids dieser Verbindung mit 10 ml 2-*m*. Eisenchlorid-Lösung behandelt, wobei das Salz unter schwacher Wärmeentwicklung fast vollständig gelöst wurde. Als das Reaktionsgemisch am folgenden Tage mit Wasser versetzt wurde, schied es eine klebrige Masse aus, welche nach dem Trocknen auf Tonscherben 0,8 g wog und bei Zusatz von Petroläther zu ihrer Lösung in Benzol nur eine Spur einer klebrigen Masse ausschied. Die davon abdekantierte Lösung lieferte bei der freiwilligen Eindunstung 0,6 g 2-Phenyl-1,3,4-thiodiazol als eine strahlig kristallinische Kruste vom Schmp. 39°–41°. — Mit besserer Ausbeute wurde dieselbe Verbindung durch Oxydation des Methylthiobenzhydrazids mit Pyridiniumtribromid erhalten. Hierbei wurden 4 g des Hydrochlorids mit einer Lösung von 7,2 g des Tribromids (vom J-Äquiv.-Gew. 179) in 50 ml gew. Alkohol versetzt, wobei unter schwacher Wärmeentwicklung im Laufe einiger Minuten eine grüngelbe Lösung entstand. Am folgenden Tage wurde diese Lösung mit 40 ml 1-*n*. Natronlauge versetzt, wonach sie bei freiwilliger Eindunstung auf 35 g Gewicht 2,4 g Kristallschuppen vom Schmp. 38°–40° ausschied. Beim Fällen mit eiskaltem Wasser aus seiner Lösung in Alkohol und Aufbewahren des Gemisches im Eisschrank lieferte das Produkt naphthalinähnliche Schuppen vom Schmp. 40°–41° (Präp. 1). — Dieselbe Verbindung wurde durch Erwärmen des Thiobenzhydrazids selbst mit Ameisensäure erhalten. Hierbei wurden 1,6 g des Thiohydrazids mit 2 g 85 %iger Ameisensäure eine Stunde auf dem Wasserbade erhitzt, wonach das flüssige Gemisch daselbst eingedunstet

¹ In ähnlicher Weise erhielten neulich YOUNG und WOOD (6) das 2-Benzylmercapto-1,3,4-thiodiazol aus Benzylthiocarbazinat und Ameisensäure.

wurde. Beim Erkalten wurden dann 1,7 g einer strahlig kristallinischen Masse vom Schmp. 37°–39° erhalten, welche bei vorsichtiger Fällung ihrer Lösung in Benzol mit Petroläther und Verwahren der entstandenen Emulsion im Eisschrank farblose Schuppen vom Schmp. 40°–40,5° lieferte (Präp. 2).

Für $C_8H_6N_2S$ (162,21):

ber.: C 59,23, H 3,73, N 17,27, S 19,77 %,

für Präp. 1 gef.: C 59,20, H 3,73, N 17,13, S 19,72 %,

für Präp. 2 gef.: C — H — N 17,20, S 19,73 %.

Bei der Darstellung des 2-Phenyl-1,3,4-thiodiazols aus dem Thiobenzhydrazon der *Glyoxylsäure* wurden zuerst gemäss HELLSTRÖM und HOLMBERG (7) 2,1 g Methylthioglykolsäure mit 20 ml 1-n. Natronlauge neutralisiert und dann unter Umrührung und Kühlung mit kaltem Wasser mit 5,5 g Kalumpersulfat oxydiert, wonach eine Lösung von 5,4 g Quecksilberchlorid in 15 ml 5-n. Salzsäure zugesetzt wurde. Nach Erhitzen des Gemisches 4 Stunden auf dem Wasserbade und Erkalten wurde das ausgeschiedene Chlorquecksilbermethylmercaptid abgesaugt, das Filtrat mit Schwefelwasserstoff gesättigt, ein wenig dabei ausgefälltes Quecksilbersulfid abfiltriert und das Filtrat mit einer Lösung von 3 g Thiobenzhydrazid in 15 ml 2-n. Salzsäure versetzt. Hierbei entstand sofort eine Emulsion, welche bald in einen Brei aus nadelförmigen Prismen überging. Nach einer Stunde wurde das so entstandene, nicht haltbare (1) Glyoxylsäure-thiobenzhydrazon abgenutscht, mit Wasser gewaschen und in 50 ml Alkohol gelöst. Die Lösung lieferte mit 20 ml 2-m. Eisenchlorid-Lösung ein braunviolett, allmählich braunes und zum Schluss gelbes Gemisch, welches bei freiwilliger Eindunstung ein klebriges Öl ausschied. Das Öl wurde mit Äther aufgenommen und die ätherische Lösung mit Wasser und verd. Natronlauge gewaschen, wonach sie bei freiwilliger Eindunstung 2,4 g einer Kristallmasse vom Schmp. 39°–41° lieferte. Nach dem Umkristallisieren wie beim Präp. 2 oben schmolz das so erhaltene 2-Phenyl-1,3,4-thiodiazol bei 40°–41° und ergab bei der Analyse:

Für $C_8H_6N_2S$ (162,21):

ber.: C 59,23, H 3,73, N 17,27, S 19,77 %,

gef.: C 59,13, H 3,71, N 17,21, S 19,80 %.

B. 5-Methyl-2-phenyl-1,3,4-thiodiazol

Da das Thiobenzhydrazon des *Azetalddehyds* bei einem früheren Versuche, es darzustellen (1), nicht zum Kristallisieren gebracht werden konnte, wurden jetzt 5 g einer 10 %igen Lösung des Aldehyds in Wasser mit 1,5 g Thiobenzhydrazid in 10 ml Alkohol versetzt und das dabei entstandene und am folgenden Tage unveränderte, homogene Gemisch dann mit einer Lösung von 2,7 g Kalumpersulfat in 50 ml Wasser versetzt, wobei eine Emulsion entstand, welche ein visköses Öl ausschied. Das Öl ging allmählich in eine weiche Kristallmasse über, welche in Alkohol gelöst wurde, wonach die Lösung bei freiwilliger Eindunstung farblose Schuppen vom Schmp. 105°–107° und der Zusammensetzung des erwarteten 5-Methyl-2-phenyl-1,3,4-thiodiazols (Präp. 1) lieferte. — Bei einem ähnlichen Versuche, aber mit 20 ml 1-m. Eisenchlorid-Lösung als Oxydationsmittel, wurden 0,4 g schwach gelbliche Schuppen vom Schmp. 95°–105° erhalten.

Das betreffende Thiodiazol entsteht auch beim Oxydieren des Thiobenzhydrazons der *Brenztraubensäure*. Diese Verbindung, welche früher (1) aus der Brenztraubensäure selbst und Thiobenzhydrazid bereitet wurde, wurde jetzt erhalten, als eine Lösung von 5,4 g des Halbmercaptols dieser Säure mit Thioglykolsäure in 20 ml Wasser mit 4,5 g Thiobenzhydrazid in 20 ml 2-*n*. Salzsäure versetzt wurde. Hierbei wurden 6,3 g Kristallpulver vom Schmp. 114°–116° erhalten, und als 2,2 g davon in 30 ml Alkohol mit 20 ml 1-*m*. Eisenchlorid-Lösung versetzt wurden, entstand ein dunkelbraunes Gemisch, welches am folgenden Tage grüngelb geworden war und dann bei der freiwilligen Eindunstung 1,6 g schmale Schuppen vom Schmp. ca. 96°–106° ausschied. Beim Umkristallisieren aus 5 ml gew. Alkohol wurden hieraus 0,9 g Schuppen (Präp. 2) vom Schmp. 107°–108° im Gemisch mit Präp. 1 106,5°–108° erhalten.

Für $C_9H_8N_2S$ (176,24):

ber.: C 61,33, H 4,58, N 15,90, S 18,19 %,

für Präp. 1 gef.: C 61,25, H 4,49, N 15,64, S 18,22 %,

für Präp. 2 gef.: C 61,42, H 4,60, N 15,88, S 18,19 %.

Als eine Lösung von 2,2 g des Brenztraubensäure-thiobenzhydrazons in 10 ml 1-*n*. Natronlauge mit 2,7 g Kaliumpersulfat in 50 ml Wasser versetzt wurde, entstand allmählich und unter schwacher Wärmeentwicklung eine Emulsion, welche bis zum folgenden Tage 1,8 g eines Gemisches aus spröden Massen und flachen Prismen ausschied. Beim Umkristallisieren aus 5 ml gew. Alkohol wurden hieraus 0,2 g farblose Schuppen vom Schmp. 106°–107° und mit 18,08, ber. 18,19, % S erhalten.

Bei Versuchen, ein Thiodiazol aus dem früher (1) beschriebenen Thiobenzhydrazon des *Azetons* darzustellen, wurde zuerst eine Lösung von 2,0 g dieses Hydrazons in 20 ml Alkohol mit 20 ml 1-*m*. Eisenchlorid-Lösung versetzt, wonach bis zum folgenden Tage 1,1 g eines auf Tonscherben getrockneten Produktes abgeschieden wurden. Beim Umkristallisieren aus 10 ml gew. Alkohol wurden hieraus 0,7 g eines Kristallgemisches erhalten, welches bei ca. 80°–135° schmolz und bei erneutem Umkristallisieren aus 10 ml Benzol 0,2 g 2,5-Diphenyl-1,3,4-thiodiazol vom Schmp. 141°–143° und mit 13,37, ber. 13,45, % S lieferte. — Auch mit Kaliumpersulfat und mit Pyridiniumtribromid lieferte das Azeton-thiobenzhydrazon nur das genannte Diphenylthiodiazol als kristallisierbares Produkt.

Behandlungen des Thiobenzhydrazons des *Azetessigesters* mit Eisenchlorid, Kaliumpersulfat oder Pyridiniumtribromid ergaben überhaupt keine kristallisierbaren Produkte. Das hierbei benutzte Thiohydrazon, welches früher nur als ein viskoses Öl erhalten worden ist (1), konnte indessen jetzt zur Kristallisation gebracht werden. Hierbei wurde eine Lösung von 3 g Thiobenzhydrazid in 10 ml gew. Alkohol mit 2,6 g des Azetessigesters in der gleichen Menge des Alkohols versetzt, wobei allmählich eine schwache Wärmeentwicklung eintrat, und als nach 2 Stunden eine Probe des Reaktionsgemisches auf dem Uhrglase verrieben wurde, trat Kristallisierung ein, wonach die mit der Probe geimpfte Reaktionslösung 4,2 g farblose, dicke Prismen vom Schmp. 78°–78,5° ausschied.

Für $C_{13}H_{16}O_2N_2S$ (264,34):

ber.: C 59,06, H 6,10, N 10,60, S 12,13 %,

gef.: C 58,94, H 6,12, N 10,56, S 12,16 %.

Die Mutterlauge des analysierten Produktes wurde von 50 ml Wasser emulgiert und setzte danach noch 0,9 g flache Nadeln vom Schmp. 77°–78,5° ab.

C. 5-Oxymethyl-2-phenyl-1,3,4-thiodiazol

Das früher als Oxydationsprodukt des Glykolaldehyd-thiobenzhydrazons, des *d*-Fructose- und des *l*-Sorbose-thiobenzhydrazids (3, S. 81–82) erhaltene 5-Oxymethyl-2-phenyl-1,3,4-thiodiazol kann auch aus dem *Dioxyazeton* in der gleichen Weise gewonnen werden. Bei einem Versuche hierüber wurde eine gemäss FISCHER und TAFEL (8) aus 10 g Glycerin bereitete Lösung von „Glycerose“ mit 4 g Natriumbisulfit und danach mit 5 g Thiobenzhydrazid in 50 ml Alkohol versetzt, wonach das Gemisch bis zum folgenden Tage nur ein wenig eines schlammartigen Pulvers absetzte. Das Filtrat davon schied bei freiwilliger Eindunstung zu 90 g Gewicht ein Öl aus, welches zu 4,1 g einer weichen, kristallinen Masse erstarrte. Nach der Behandlung dieser Masse mit 30 ml 1-*n*. Salzsäure und 50 ml Äther lieferte die ätherische Schicht bei der freiwilligen Eindunstung 3 g einer Kristallmasse, welche nach der Umkristallisierung aus 50 ml Benzol 2,5 g wog und bei 103°–105° schmolz. Nach Umkristallisierung aus wenig abs. Alkohol bestand das Produkt aus farblosen, flachen und schmalen Prismen vom Schmp. 104°–106° und der Zusammensetzung des Dioxyazetonthiobenzhydrazons.

Für $C_{10}H_{12}O_2N_2S$ (224,28):

ber.: C 53,55, H 5,39, N 12,49, S 14,30 %,

gef.: C 53,76, H 5,45, N 12,45, S 14,28 %.

Eine Lösung von 2,3 g aus Benzol umkristallisierten Dioxyazeton-thiobenzhydrazons in 20 ml Alkohol lieferte mit 10 ml 2-*m*. Eisenchlorid-Lösung unter schwacher Wärmeentwicklung ein dunkelgrünes Gemisch, welches am folgenden Tage gelbbraun geworden war und dann bei der freiwilligen Eindunstung ein viskoses Öl ausschied. Nach Impfen mit aus dem Thiobenzhydrazid der *d*-Fructose gewonnenem Oxymethyl-phenylthiodiazol ging dieses Öl in 1,2 g einer etwas teigigen Kristallmasse über, welche nach Umkristallisierungen zuerst aus Alkohol und dann aus Benzol aus 0,5 g farblose Schuppen vom Schmp. 101°–102° bestand.

Für $C_9H_8ON_2S$ (192,24):

ber.: C 56,23, H 4,19, N 14,57, S 16,68 %,

gef.: C 56,27, H 4,24, N 14,57, S 16,61 %.

D. 2,5-Diphenyl-1,3,4-thiodiazol

Wie mit Eisenschlorid (1) ergibt das *Benzaldehyd-thiobenzhydrazon* auch mit Wasserstoffsuperoxyd das 2,5-Diphenyl-1,3,4-thiodiazol. Bei einem Versuche hierüber lieferte eine Lösung von 2,7 g des Hydrazons in 25 ml Azeton mit 2 g Perhydro ein homogenes, am folgenden Tage anscheinend unverändertes Gemisch, welches bei freiwilliger Eindunstung und Zusatz von etwas Wasser 3,2 g teigige Massen ausschied. Bei der Umkristallisierung aus 20 ml gew. Alkohol wurden hieraus 1,0 g farblose Schuppen vom Schmp. 139°–141° erhalten, und nach noch einer Umkristallisierung in der gleichen Weise wog das Produkt 0,7 g, schmolz bei 142°–143° und zeigte die Zusammensetzung des Diphenylthiodiazols.

Für $C_{14}H_{10}N_2S$ (238,30):

ber.: C 70,56, H 4,23, N 11,76, S 13,45 %,

gef.: C 70,30, H 4,20, N 11,70, S 13,48 %.

Bei Oxydationen mit Kaliumpersulfat in alkalischen Lösungen lieferte aber das Benzaldehyd-thiobenzhydrazon das Disulfid der *iso*-Form des Thiohydrazons. Bei dem ersten Versuche hierüber wurde eine Lösung von 2,4 g des Thiohydrazons in 20 ml 0,5-*n*. Natronlauge mit einer Lösung von 2,7 g Kaliumpersulfat in 50 ml Wasser versetzt, wobei ein klares Gemisch erhalten wurde. Dieses wurde schnell emulgiert und schied bis zum folgenden Tage 2 g spröde, gelbliche Massen vom Schmp. ca. 110°–118° aus, welche bei der Umkristallisierung aus 75 ml gew. Alkohol 0,9 g schmale, flache Prismen vom Schmp. 129°–131° bei langsamer und 134°–136° bei schneller Erhitzung lieferten (Präp. 1). — Bei einem neuen Versuche in der gleichen Weise, aber mit 20 ml 1-*n*. Natronlauge als Lösungsmittel für das Thiohydrazon, wurden 2,0 g eines feinkristallinen, gelben Pulvers vom Schmp. 110°–115° erhalten. Als dieses Produkt aus 100 ml gew. Alkohol umkristallisiert wurde, lieferte es 1,4 g goldgelbe Prismen oder schmale Täfelchen, welche bei 118°–119°, im Gemisch mit dem Präp. 1 bei 116°–119° (Schmelze opak, klar bei ca. 125°) schmolzen (Präp. 2).

Für $C_{28}H_{22}N_4S_2$ (478,62):

ber.: C 70,26, H 4,63, N 11,71, S 13,40 %,

für Präp. 1 gef.: C 70,30, H 4,60, N 11,79, S 13,48 %,

für Präp. 2 gef.: C 70,40, H 4,57, N 11,73, S 13,42 %.

Ein Thiobenzhydrazon des Azetophenons konnte bei früher angestellten Versuchen (1) nicht erhalten werden, und dies war jetzt auch nicht der Fall mit dem *Benzoylcarbinol*. Dagegen gelang die Darstellung des entsprechenden Derivates des *Phenazylazetates*. Bei einem Versuche hierüber wurde eine Lösung von 11,8 g des Azetates in 75 ml gew. Alkohol mit 10 g Thiobenzhydrazid versetzt, wobei 90 ml einer klaren Lösung erhalten wurden. Von der noch am folgenden Tage anscheinend unveränderten Lösung wurden 67 ml, 1/20 Molen entsprechend, mit 10 ml 1-*n*. Natronlauge versetzt, wobei sie zuerst emulgiert, aber bald wieder klar wurde und danach bis zum folgenden Tage 12 g Prismen vom Schmp. 138°–140° in offenem und 119°–122° in zugeschmolzenem Röhrchen ausschied (Präp. 1). — Von derselben Reaktionslösung wurden 10 ml mit 2 ml 2-*n*. Salzsäure versetzt, wobei eine Emulsion entstand, welche ein viskoses Öl ausschied. Dieses ging bis zum folgenden Tage in 0,9 g flache, farblose Prismen über, welche wie oben bei 137°–139° bzw. 118°–119° schmolzen (Präp. 2).

Für $C_{17}H_{16}O_2N_2S$, $H_2O = C_{17}H_{18}O_3N_2S$ (330,40):

ber.: C 61,79, H 5,49, N 8,48, S 9,71 %,

für Präp. 1 gef.: C 61,70, H 5,41, N 8,53, S 9,72 %,

für Präp. 2 gef.: C 61,55, H 5,44, N 8,54, S 9,68 %.

Bei der Umkristallisierung von 2 g des Präp. 1 aus 10 ml gew. Alkohol wurden 1,65 g farblose, flache Prismen erhalten, welche wie oben bei 139°–141° bzw. 121°–123° schmolzen und beim Trocknen im Vakuum über Phosphorsäureanhydrid 5,27, ber. 5,45, % an Gewicht verloren (dann Präp. 3). Von demselben Präp. ergaben 2 g bei der Umkristallisierung aus 10 ml abs. Alkohol 1,5 g Prismen vom Schmp. 139°–141° (Präp. 4).

Für $C_{17}H_{16}O_2N_2S$ (312,38):

ber.: C 65,36, H 5,16, N 8,97, S 10,26 %,

für Präp. 3 gef.: C 65,12, H 5,13, N — S —

für Präp. 4 gef.: C — H — N 8,99, S 10,26 %.

Behandlungen des Phenazylazetat-thiobenzhydrazons mit Kaliumpersulfat, Eisenchlorid und Pyridiniumtribromid lieferten keine definierbaren Produkte.

Als eine Lösung von 8 g *Phenazylbromid* in 50 ml gew. Alkohol mit 6,1 g Thiobenzhydrazid in 20 ml desselben Alkohols versetzt wurde, entstand ein homogenes Gemisch, welches nach einigen Minuten Kristalle abzuschneiden begann und nach 3 Stunden beim Absaugen 7 g gelbliche, dünne Schuppen vom Schmp. 200°–203° (nach schwacher Sinterung bei ca. 130°) lieferte (Präp. 1). Beim Umkristallisieren von 2 g des Produktes aus 20 ml gew. Alkohol wurden 1,6 g cremefarbene Schuppen oder dünne Täfelchen vom Schmp. 205°–207° erhalten (Präp. 2). Beide Präparate zeigten die Zusammensetzung eines Diphenylthiopyridazins.

Für $C_{15}H_{12}N_2S$ (252,33):

ber.: C 71,39, H 4,79, N 11,10, S 12,71 %,
für Präp. 1 gef.: C 71,20, H 4,83, N 11,07, S 12,72 %,
für Präp. 2 gef.: C 71,59, H 4,75, N 11,18, S 12,66 %.

Eine Lösung von 4,2 g *Benzil* in 25 ml warmem, gew. Alkohol ergab mit 3 g Thiobenzhydrazid in 15 ml desselben Lösungsmittels ein homogenes Gemisch, welches bis zum folgenden Tage ein klebriges Öl ausschied. Beim Reiben ging dieses Öl in 6,1 g schmale, schwach gelbliche Prismen über, welche bei 118°–120° schmolzen. Bei der Umkristallisierung aus 26 ml gew. Alkohol lieferten 2,6 g dieses Produktes 2,3 g Benzil-monothiothiobenzhydrazon als schmale, gelbe Prismen vom Schmp. 116°–119°.

Für $C_{21}H_{16}ON_2S$ (344,42):

ber.: N 8,13, S 9,31 %; gef.: N 8,18, S 9,34 %.

Als eine warme Lösung von 3,5 g des Rohproduktes in 50 ml Alkohol mit 10 ml 2-m. Eisenchlorid-Lösung versetzt wurde, entstand ein dunkelgraugrünes Gemisch, welches bis zum folgenden Tage 1,6 g farblose Schuppen vom Schmp. 139°–141° nebst 0,8 g mechanisch von diesen herauslesbare, harzige Massen ausschied. Das kristallisierte Produkt wurde aus 10 ml Benzol umkristallisiert und lieferte dabei 1,0 g 2,5-Diphenyl-1,3,4-thiodiazol als farblose Schuppen vom Schmp. 141°–143°.

Für $C_{14}H_{10}N_2S$ (238,30):

ber.: N 11,76, S 13,45 %; gef.: N 11,71, S 13,32 %.

Die Mutterlaugen des rohen und des umkristallisierten Produktes lieferten bei der freiwilligen Eindunstung teigige Massen, welche mit Soda-Lösung behandelt wurden, wonach das Filtrat von etwas ungelöstem Materiale mit Salzsäure 0,1 g Benzoessäure vom Schmp. 122°–123° und Äquiv.-Gew. 121,9 ausschied.

E. 2-Phenyl-5-guajacyl-1,3,4-thiodiazol

Aus 5 g *Vanillin* und 5 g Thiobenzhydrazid in je 20 ml gew. Alkohol wurde ein homogenes Gemisch erhalten, welches mit bei einem Vorversuche erhaltenen Kriställchen geimpft wurde, wonach es bis zum folgenden Tage 9 g Vanillin-thiobenzhydrazon als dünne Täfelchen vom Schmp. 141°–143° ausschied. Beim Umkristalli-

sieren von 4,3 g dieses Produktes aus 40 ml gew. Alkohol wurden 3,8 g gelbliche, Täfelchen vom Schmp. 142° – 143° erhalten.

Für $C_{15}H_{14}O_2N_2S$ (286,34):

ber.: C 62,91, H 4,93, N 9,78, S 11,20, OCH_3 10,83 %,

gef.: C 62,92, H 4,81, N 9,83, S 11,15, OCH_3 10,72 %.

Als 2,9 g des Rohproduktes in 35 ml warmem Alkohol mit 25 ml 1-*m.* Eisenchlorid-Lösung versetzt wurden, trat eine schwache Gasentwicklung ein, und ein braunes, trübes Gemisch entstand, wonach bis zum folgenden Tage eine unbedeutende Menge eines klebrigen Pulvers abgeschieden wurde. Das Filtrat davon lieferte bei der freiwilligen Eindunstung 1,5 g gelbe Nadeln vom Schmp. ca. 160° – 170° , welche bei der Umkristallisierung aus 40 ml gew. Alkohol 1,1 g kleine Prismen vom Schmp. 170° – 180° lieferten. Nach noch einer Umkristallisierung in der gleichen Weise bestand das Produkt aus farblosen Prismen vom Schmp. 178° – 180° und mit der Zusammensetzung des erwarteten Thiodiazols.

Für $C_{15}H_{12}O_2N_2S$ (284,33):

ber.: C 63,36, H 4,25, N 9,85, S 11,28, OCH_3 10,91 %,

gef.: C 63,54, H 4,23, N 9,91, S 11,27, OCH_3 10,74 %.

Aus 3,3 g *Azetovanillon*, 3,1 g Thiobenzhydrazid und 20 ml gew. Alkohol entstand in gelinder Wärme eine klare, gelbgrüne Lösung, welche nach 8 Stunden mit einem kristallisierten Eindunstungsrest einer Probe des Reaktionsgemisches geimpft wurde, wonach bis zum folgenden Tage 1,9 g gelbe, flache Prismen oder schmale, schiefwinklige Täfelchen vom Schmp. 124° – $125,5^{\circ}$ ausgeschieden wurden (Präp. 1). Das Filtrat davon lieferte bei freiwilliger Eindunstung noch 1,7 g Kristalle vom Schmp. 124° – 126° , nach der Umkristallisierung aus 10 ml gew. Alkohol 1,25 g vom Schmp. 125° – $126,5^{\circ}$ (Präp. 2). Gemäss den Analysen bestanden die Präparate aus dem *bis*-thiobenzhydrazid des Azetovanillons.

Für $C_{23}H_{24}O_2N_4S_2$ (452,59):

ber.: C 61,03, H 5,35, N 12,38, S 14,17, OCH_3 6,85 %,

für Präp. 1 gef.: C 61,23, H 5,41, N 12,25, S — OCH_3 —

für Präp. 2 gef.: C 61,02, H 5,32, N 12,34, S 14,14, OCH_3 6,94 %.

F. 2-Phenyl-5-dimethoxyphenyl-1,3,4-thiodiazol

Aus einer Lösung von 3,3 g *Veratrumaldehyd* in 20 ml gew. Alkohol und 3 g Thiobenzhydrazid entstand ein homogenes Gemisch, welches nach ca. 10 Minuten Kristalle abzuschneiden begann und bis zum folgenden Tage 5,7 g Thiohydrazon als fast nadelförmige, gelbe Prismen vom Schmp. 125° – 127° lieferte.

Für $C_{16}H_{16}O_2N_2S$ (300,37):

ber.: N 9,33, S 10,67 %; gef.: N 9,37, S 10,68 %.

Eine Lösung von 2 g des Thiohydrazons in 40 ml warmem Alkohol wurde mit 5 ml 2-*m.* Eisenchlorid-Lösung versetzt, wobei ein dunkelbraunes Gemisch entstand, welches bis zum folgenden Tage 1,1 g eines Gemisches von Kristallen mit harzigen

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Massen ausschied. Mechanisch herausgelesene Kristalle schmolzen bei 142° – 147° , und als das Totalprodukt aus 10 ml gew. Alkohol umkristallisiert wurde, wurden 0,8 g schwarzbraune, kleine Prismen vom Schmp. 144° – 147° erhalten. Beim neuen Umkristallisieren aus 5 ml Benzol wurden hieraus 0,4 g Phenyl-dimethoxyphenylthiodiazol als farblose, schmale Prismen vom Schmp. 149° – 150° erhalten.

Für $C_{16}H_{14}O_2N_2S$ (298,35):

ber.: C 64,41, H 4,73, N 9,39, S 10,75 %,

gef.: C 64,39, H 4,91, N 9,49, S 10,64 %.

Eine Lösung von 3,6 g *Azetoveratron* in 20 ml warmem, gew. Alkohol lieferte mit 3,1 g Thiobenzhydrazid in 10 ml desselben Lösungsmittels ein homogenes Gemisch, welches am folgenden Tage nichts ausgeschieden hatte, aber dann bei der freiwilligen Eindunstung 5,2 g einer pomeranzenfarbenen Kristallmasse vom Schmp. 130° – 131° lieferte. Gemäss der Analyse wurde hier das einfache Thiobenzhydrazon erhalten.

Für $C_{17}H_{18}O_2N_2S$ (314,40):

ber.: C 64,94, H 5,77, N 8,91, S 10,20 %,

gef.: C 64,91, H 5,77, N 8,93, S 10,23 %.

Als eine warme Lösung von 3,1 g des *Azetoveratron*-thiobenzhydrazons in 40 ml Alkohol mit 20 ml 1-*m.* Eisenchlorid-Lösung versetzt wurde, entstand unter heftiger Gasentwicklung eine zuerst graugrüne, dann braungüne Emulsion, welche dunkelgefärbte, teigige Massen ausschied. Nach dem Trocknen auf Tonscherben wogen diese 1,5 g, und bei der weiteren Bearbeitung konnte nichts einheitliches daraus gewonnen werden.

3. Oxydationsprodukte einiger Thiosemicarbazone

A. 5-Methyl-2-amino-1,3,4-thiodiazol

Als 10 ml einer 10 %igen, wässrigen Lösung von *Azetalddehyd* mit einer 50° warmen Lösung von 1,8 g Thiosemicarbazid in 40 ml 50 %igem Alkohol versetzt wurde, entstand ein homogenes Gemisch, welches sich bis zum folgenden Tage nur schwach trübte und dann nach Filtrierung mit 20 ml einer 2-*m.* Eisenchlorid-Lösung versetzt wurde. Dabei entstand ein violettbraunes, am folgenden Tage rotbraunes Gemisch, welches bei der freiwilligen Eindunstung zu 26 g Gewicht 1,7 g schmale Prismen ausschied. Das Produkt ging in zugeschmolzenem Röhrchen bei 105° – 108° in eine blasige Schmelze über und zeigte (Phenolphthalein) das Äquiv.-Gew. 164,3, ber. 169,6. Beim Umkristallisieren von 1,6 g davon aus 10 ml gew. Alkohol wurden 0,9 g farblose, flache Prismen erhalten, welche in offenem Röhrchen bei 105° – 107° , in zugeschmolzenem bei 109° – 110° (wie oben) schmolzen. Im Vakuum über Phosphorsäureanhydrid verlor das Präparat 10,65, für 1 H_2O ber. 10,62, % an Gewicht, schmolz danach bei 148° – 150° und ergab bei der Analyse:

Für $C_3H_6N_3ClS$ (151,62):

ber.: Cl 23,39 %, Äquiv.-Gew. 151,6; gef. Cl 23,51 %, Äquiv.-Gew. 152,4.

Das dem so erhaltenen Hydrochlorid entsprechende 5-Methyl-2-amino-1,3,4-thiodiazol wurde erhalten, als die Mutterlauge des oben umkristallisierten Hydrochlorids mit 5,5 ml 1-*n*. Natronlauge versetzt wurde. Hierbei entstand zuerst eine unbedeutende Menge eines flockigen Niederschlages, wonach das Filtrat davon bei der freiwilligen Eindunstung 0,3 g dicke, glasklare Prismen vom Schmp. 221°–223° absetzte.

Für $C_3H_5N_3S$ (115,16):

ber.: C 31,29, H 4,38, N 36,49, S 27,84 %.

gef.: C 31,33, H 4,34, N 36,61, S 27,85 %.

Dasselbe Thiodiazol wurde auch aus den Thiosemicarbazonen des *Oxyazetons* und des *Methyl- α -oxyäthylketons* bei den Oxydationen mit Eisenchlorid erhalten. Hierbei wurde zuerst eine Lösung von 2,7 g Thiosemicarbazid in 60 ml warmem, 50 %igem Alkohol mit 2,7 g Oxyazeton versetzt, wonach bald kleine, dicke, flächenreiche Prismen ausgeschieden wurden. Die Ausbeute an dem am folgenden Tage abgenutzten Produkte vom Schmp. 172°–173° (unter Aufschäumen) betrug 4,1 g, und aus der Analyse geht hervor, das es aus dem erwarteten Thiosemicarbazon bestand.

Für $C_4H_9ON_3S$ (147,20):

ber.: C 32,64, H 6,16, N 28,55, S 21,78 %.

gef.: C 32,73, H 6,17, N 28,51, S 21,67 %.

Beim Schütteln von 3,0 g des Oxyazeton-thiosemicarbazons mit 30 ml Alkohol und 20 ml 2-*m*. Eisenchlorid-Lösung entstand schnell eine braunviolette Lösung, welche bei der freiwilligen Eindunstung zu 18 g Gewicht 1,8 g eines Kristallpulvers vom Schmp. ca. 90° (in zugeschmolzenem Röhrchen) ausschied. Als eine wässrige Lösung von diesem Produkte mit Natronlauge neutralisiert wurde, entstand ein Niederschlag, welcher beim Umkristallisieren aus Alkohol 5-Methyl-2-amino-1,3,4-thiodiazol als farblose, flache Prismen vom Schmp. 221°–223° lieferte.

Für $C_3H_5N_3S$ (115,16):

ber.: C 31,29, H 4,38 %; gef.: C 31,03, H 4,10 %.

Als eine Lösung von 1,8 g Thiosemicarbazid in 40 ml warmem, 50 %igem Alkohol mit 2 g Methyl- α -oxyäthylketon versetzt wurde, entstand ein homogenes Gemisch, welches am folgenden Tage und auch nach 2stündiger Erhitzung auf dem Wasserbade nichts ausschied, aber dann bei freiwilliger Eindunstung 2 g Thiosemicarbazon als eine Kristallkruste vom Schmp. 160°–161° lieferte.

Für $C_5H_{11}ON_3S$ (161,23):

ber.: N 26,07, S 19,89 %; gef. N 26,20, S 19,99 %.

Von diesem Thiosemicarbazon wurden 1,6 g in 25 ml Alkohol gelöst, wonach 12 ml 2-*m*. Eisenchlorid-Lösung zugesetzt wurden. Hierbei entstand ein violettbraunes, am folgenden Tage braunes Gemisch, welches bei freiwilliger Eindunstung auf 12 g Gewicht 1,0 g Prismen vom Schmp. 97°–99° (in zugeschmolzenem Röhrchen) ausschied. Aus diesem Produkte wurde mit Natronlauge wie oben schmale Täfelchen vom Schmp. 223°–224° und der Zusammensetzung des 5-Methyl-2-amino-1,3,4-thiodiazols erhalten.

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Für $C_8H_5N_3S$ (115,16):

ber.: C 31,29, H 4,38, N 36,49, S 27,84 %,

gef.: C 31,29, H 4,43, N 36,39, S 27,71 %.

Ein Versuch, das 5-Methyl-2-amino-1,3,4-thiodiazol durch Oxydation des Thiosemicarbazons des *Azetons* mit Eisenchlorid darzustellen, gelang nicht, denn dabei wurden nur teigige Massen erhalten, aus denen nichts Kristallisierbares isoliert werden konnte.

B. 5-*l*-Arabinotetraoxybutyl-2-amino-1,3,4-thiodiazol

Beim Erhitzen einer Lösung von 3 g *l*-Arabinose in 20 ml 50 %igem Alkohol mit 1,8 g Thiosemicarbazid entstand eine klare Lösung, welche beim Erkalten nur schwach emulgiert wurde, nach Filtrierung und bis zum folgenden Tage aber 3,4 g glasklare, flächenreiche, dicke Prismen vom Schmp. 148° – 150° (Schmelze gelb und blasig) ausschied.

Für $C_6H_{13}O_4N_3S$, $H_2O = C_6H_{15}O_5N_3S$ (241,27):

ber.: C 29,87, H 6,27, N 17,42, S 13,29, H_2O 7,47 %,

gef.: C 29,92, H 6,21, N 17,44, S 13,24, H_2O 7,53 %.¹

Von der getrockneten Probe zeigten 0,3154 g in Wasser auf 20,04 ml Volumen gelöst 15 und 300 Minuten nach dem Zusatz des Wassers $2\alpha_D^{20} = +0,88^{\circ}$, $[\alpha]_D^{20} = +28,0^{\circ}$ und $[M]_D^{20} = +62,4^{\circ}$.

Eine Lösung von 2,2 g des erhaltenen *l*-Arabinose-thiosemicarbazons in 20 ml Wasser ergab mit 10 ml 2-*m*. Eisenchlorid-Lösung ein rotbraunes Gemisch, welches bei der freiwilligen Eindunstung zu 18 g Gewicht 1,6 g Kristalle vom Schmp. 197° – 199° (unter Gasentwicklung nach Braunfärbung) ausschied. Bei der Umkristallisierung aus 2 ml Wasser wurden hieraus 0,8 g farblose, flache Prismen oder dicke, schmale Täfelchen vom Schmp. 198° – 200° (wie vorher) erhalten.

Für $C_6H_{12}O_4N_3ClS$ (257,70):

ber.: C 27,96, H 4,69, Cl 13,76 %, Äquiv.-Gew. 257,7,

gef.: C 28,18, H 4,80, Cl 13,81 %, Äquiv.-Gew. 259,1.

Eine Lösung von 0,4192 g des analysierten Hydrochlorids in Wasser auf 10,04 ml Volumen zeigte 10 und 500 Minuten nach dem Zusatz des Wassers $\alpha_D^{20} = +0,46^{\circ}$, $[\alpha]_D^{20} = +11,0^{\circ}$ und $[M]_D^{20} = +28,4^{\circ}$.

Die wässrige Mutterlauge des umkristallisierten Hydrochlorids wurde mit 6 ml 1-*n*. Natronlauge neutralisiert, wonach sie schnell in einen Brei aus farblosen, fast haarfeinen Nadeln vom Schmp. 191° – 193° (Zersetzung) überging.

Für $C_6H_{11}O_4N_3S$ (221,24):

ber.: C 32,57, H 5,01 %; gef.: C 32,29, H 4,98 %.

Eine in gelinder Wärme bereitete Lösung von 0,4091 des freien 5-*l*-Arabinotetraoxybutyl-2-amino-1,3,4-thiodiazols in Wasser auf 10,04 ml Volumen zeigte unmittel-

¹ Gewichtsverlust im Vakuum über Phosphorsäureanhydrid.

bar nach der Temperierung sowie 2 Stunden danach $\alpha_D^{20} = +0,07^\circ$, $[\alpha]_D^{20} = +1,7^\circ$ und $[M]_D^{20} = +3,8^\circ$.

Das Hydrochlorid des 5-*d*-Arabinotetraoxybutyl-2-amino-1,3,4-thiodiazols wurde aus der *d*-Fructose dargestellt. Hierbei wurde eine Lösung von 3,6 g dieses Zuckers in 20 ml 50%igem Alkohol auf dem Wasserbade mit 1,8 g Thiosemicarbazid versetzt, wonach die entstandene Lösung beim Erkalten nur 0,5 g des Thiosemicarbazids ausschied. Die Erhitzung wurde daher 12 Stunden fortgesetzt, wonach die dann gelbe Lösung beim Erkalten sich nur schwach trübte und dann bei freiwilliger Eindunstung auf 9 g Gewicht 0,6 g eines heterogenen Kristallgemisches ausschied. Das Filtrat davon ergab mit 25 ml Wasser und 20 ml 2-*m*. Eisenchlorid-Lösung ein dunkelvioletttes Gemisch, welches sich schwach erwärmte und bis zum folgenden Tage eine Spur eines schlammartigen Pulvers ausschied. Das Filtrat davon lieferte bei der freiwilligen Eindunstung auf 17 g Gewicht 2,8 g Kristallpulver, welches bei der Umkristallisierung aus 5 ml Wasser 0,5 g 5-*d*-Arabinotetraoxybutyl-2-amino-1,3,4-thiodiazol-hydrochlorid vom Schmp. 198° – 200° (Zersetzung) lieferte.

Für $C_6H_{12}O_4N_3ClS$ (257,70):

ber.: N 16,31, Cl 13,76 %; gef.: N 16,54, Cl 13,66 %.

Von diesem Hydrochlorid zeigten 0,4110 g in Wasser auf 10,00 ml Volumen gelöst $\alpha_D^{20} = -0,42^\circ$, $[\alpha]_D^{20} = -10,2^\circ$ und $[M]_D^{20} = -26,3^\circ$.

C. Cyklohexanol-1-on-2-thiosemicarbazon

Von gemäss BERGMANN und GIERTH (9) hergestelltem *Cyclohexanol-1-on-2* vom Siedep.₁₃ = 80° – 81° wurden 2,3 g zu einer warmen Lösung von 1,8 g Thiosemicarbazid in 20 ml 50%igem Alkohol gesetzt, wobei eine Lösung entstand, welche bald Kristalle abzuscheiden begann und bis zum folgenden Tage 3,6 g schwach gelbliche, kleine Prismen vom Schmp. 178° – 179° (unter heftiger Gasentwicklung) lieferte.

Für $C_7H_{13}ON_3S$ (187,26):

ber.: C 44,89, H 7,00, N 22,44, S 17,12 %.

gef.: C 45,04, H 7,06, N 22,49, S 17,10 %.

Die Umsetzung dieses Thiosemicarbazons mit Eisenchlorid lieferte keine definierbaren Produkte.

D. 5-Phenyl-2-amino-1,3,4-thiodiazol

Als eine Lösung von 1,8 g *Benzaldehyd-thiosemicarbazon* vom Schmp. 162° – 163° ¹ in 25 ml warmem Alkohol mit 20 ml 1-*m*. Eisenchlorid-Lösung versetzt wurde, entstand ein dunkelbraunes Gemisch, welches allmählich schwacher gefärbt und etwas getrübt wurde. Bis zum folgenden Tage wurde eine Spur eines Pulvers abgeschieden, und das braungelbe Filtrat davon lieferte bei freiwilliger Eindunstung 1,6 g chlorwasserstoffsäures 5-Phenyl-2-amino-1,3,4-thiodiazol als eine Kristallkruste vom Schmp. ca. 205° – 210° . Beim Umkristallisieren aus 5 ml gew. Alkohol wurden hieraus sehr schwach gelbliche Krusten von flächenreichen, dicken Prismen vom Schmp. 212° – 214° ² erhalten.

¹ YOUNG und EYRE (2) fanden für das Benzaldehyd-thiosemicarbazon den Schmp. 159° – 160° .

² YOUNG und EYRE (2) fanden 213° – 214° .

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Für $C_8H_8N_3ClS$, $H_2O = C_8H_{10}ON_3ClS$ (231,71):

ber.: C 41,46, H 4,35, N 18,14, Cl 15,30, S 13,84 %,

gef.: C 41,64, H 4,35, N 18,38, Cl 15,10, S 13,83 %.

Als eine Lösung von 3 g *Benzoylcarbinol* in 10 ml warmem Alkohol mit 1,8 g Thiosemicarbazid in 20 ml ebenfalls warmem, 50 %igem Alkohol versetzt wurde, entstand ein homogenes Gemisch, welches bis zum folgenden Tage 3,4 g Thiosemicarbazon als farblose, flächenreiche, dicke Prismen vom Schmp. 170° – 171° (unter Gasentwicklung in einer gelbbraunen Schmelze) absetzte.

Für $C_9H_{11}ON_3S$ (209,27):

ber.: C 51,65, H 5,30, N 20,08, S 15,32 %,

gef.: C 51,73, H 5,34, N 20,09, S 15,32 %,

Von diesem Thiosemicarbazon lieferten 2,8 g in 50 ml warmem Alkohol mit 15 ml 2-m. Eisenchlorid-Lösung ein dunkelbraunes, bald heller gefärbtes Gemisch, welches bei der freiwilligen Eindunstung zu 19 g Gewicht 2,4 g kristallisiertes 5-Phenyl-2-amino-1,3,4-thiodiazol-hydrochlorid vom Schmp. ca. 195° – 200° (unter Zersetzung) ausschied. Nach der Umkristallisierung aus wenig Alkohol schmolz das Produkt bei 210° – 212° (Zersetzung) und enthielt 15,19, ber. 15,30, % Cl. Als eine Lösung von 0,9 g dieses Salzes in 10 ml Wasser mit 4 ml 1-n. Natronlauge neutralisiert wurde, schied sie 0,6 g der entsprechenden Base als feinkristallinisches, weisses Pulver vom Schmp. 222° – 224° ¹ aus.

Für $C_8H_7N_3S$ (177,23):

ber.: C 54,21, H 3,98, N 23,71, S 18,09 %,

gef.: C 54,04, H 3,94, N 23,78, S 17,98 %.

Aus einem Gemisch aus 10,6 g *Benzoin* in 100 ml warmem Alkohol und 4,6 g Thiosemicarbazid in 25 ml 2-n. Salzsäure wurden bis zum folgenden Tage 11,8 g Thiosemicarbazon als schmale, farblose Prismen vom Schmp. 176° – 178° ausgeschieden.

Für $C_{15}H_{15}ON_3S$ (285,36):

ber.: C 63,13, H 5,30, N 14,73, S 11,24 %,

gef.: C 63,25, H 5,27, N 14,74, S 11,21 %.

Eine Lösung von 2,9 g dieses Produktes in 25 ml warmem Alkohol lieferte mit 20 ml 1-m. Eisenchlorid-Lösung ein violettbraunes Gemisch, welches bald braungelb und milchig wurde und nach Benzaldehyd roch. Am folgenden Tage wurde eine Spur eines weissen Pulvers abfiltriert, wonach die Lösung bei freiwilliger Eindunstung 2,2 g 5-Phenyl-2-amino-1,3,4-thiodiazol-hydrochlorid als eine kristallinische Kruste vom Schmp. ca. 190° (Zersetzung) absetzte. Nach der Umkristallisierung aus gew. Alkohol schmolz das Produkt bei 213° – 215° (Zersetzung) und ergab bei der Analyse:

Für $C_8H_{10}ON_3ClS$ (231,71):

ber.: C 41,46, H 4,35, N 18,14, Cl 15,30, S 13,84 %,

gef.: C 41,72, H 4,14, N 18,13, Cl 15,06, S 13,32 %.

¹ YOUNG und EYRE (2) fanden 222° – 223° .

Beim Titrieren von 0.2991 g Sbst. in 10 ml Alkohol mit Phenolphthalein und 0,1000-n. Natronlauge wurden 13.00 ml der Lauge verbraucht, Äquiv.-Gew. ber. 231,7, gef. 230,1, wonach die Lösung bei freiwilliger Eindunstung 0.2 g freies Phenylaminthiodiazol vom Schmp. 224°–225° ausschied.

Für $C_8H_7N_3S$ (177,23):

ber.: N 23,71, S 18,09 %; gef.: N 23,59, S 18,01 %.

Aus 3 g *Phenylglyoxylsäure* und 1,8 g Thiosemicarbazid in 40 ml warmem, 50 %igem Alkohol wurde ein homogenes Gemisch erhalten, welches bis zum folgenden Tage 3,3 g Thiosemicarbazon als Kristallwarzen vom Schmp. 174°–176°¹ ausschied.

Für $C_9H_9O_2N_3S$ (223,25):

ber.: N 18,82, S 14,36 %, Äquiv.-Gew. 223,3,

gef.: N 18,87, S 14,40 %, Äquiv.-Gew. 224,2.

Eine Lösung von 2,4 g dieses Thiosemicarbazons in 25 ml warmem Alkohol lieferte mit 11 ml 2-m. Eisenchlorid-Lösung ein violettbraunes Gemisch, welches bis zum folgenden Tage ein wenig eines heterogenen Kristallpulvers ausschied. Das Filtrat davon ergab bei freiwilliger Eindunstung auf 15 g Gewicht 2 g unreines Hydrochlorid des 5-Phenyl-2-amino-1,3,4-thiodiazols vom Schmp. ca. 208°–213° (Zersetzung) und Äquiv.-Gew. 200. Bei der Titrierung abgeschiedenes Kristallpulver bestand aus der dem Hydrochlorid entsprechenden, freien Base vom Schmp. 225°–227°.

Für $C_8H_7N_3S$ (177,23):

ber.: C 54,21, H 3,98 %; gef.: C 54,17, H 3,98 %.

E. Thiosemicarbazone des Phenylglyoxals, des Propiophenons und des Phenylazetons

Aus 2,6 g gemäss dem Verf. (11) dargestelltem *Phenylglyoxal* und 1,8 g Thiosemicarbazid in 40 ml warmem, 50 %igem Alkohol wurde ein homogenes Gemisch erhalten, welches bald gelbe Kristallwarzen abzuscheiden begann. Das am folgenden Tage abgenutzte Produkt wog 2,9 g, schmolz bei 152°–154° (Zersetzung) und löste sich nicht vollständig in 30 ml heissem, gew. Alkohol. Das Filtrat von 0,5 g ungelöstem Pulver vom Schmp. 216°–218° lieferte beim Erkalten und bis zum folgenden Tage 1,7 g Thiosemicarbazon als gelbe, flache Prismen vom Schmp. 156°–158°² und mit 20,45, ber. 20,28, % N. Als eine Lösung von 2,1 g solcher Produkte in 30 ml warmem Alkohol mit 10 ml 2-m. Eisenchlorid-Lösung versetzt wurde, entstand ein schwarzgrünes, am folgenden Tage braunes Gemisch, welches bei der freiwilligen Eindunstung zu 14 g Gewicht 2,3 g Kristalle vom Schmp. 194°–197° (Zersetzung) abschied. Nach der Umkristallisierung aus Alkohol bestand das Produkt aus dicken, flächenreichen, braungelben Prismen vom Schmp. wie vorher und von der Zusammensetzung des chlorwasserstoffsäuren 5-Benzoyl-2-amino-1,3,4-thiodiazols.

¹ CORSON, SANBORN und ESS (10) haben den Schmp. des Phenylglyoxylsäure-thiosemicarbazons bei 188°–189° (Zersetzung) angegeben.

² Für ein als Acetophenon-azothioformamid, $C_6H_5COCH_2 \cdot N : N \cdot CS \cdot NH_2$, formuliertes Produkt haben WOLFF und LINDENHAYN (12) den Schmp. 170° angegeben.

B. HOLMBERG, *Oxydationsprodukte einiger Thiobenzhydrazone*

Für $C_9H_8ON_3ClS$ (241,70):

ber.: C 44,72, H 3,34, N 17,39, Cl 14,67, S 13,27 %.

gef.: C 44,77, H 3,29, N 17,46, Cl 14,57, S 13,32 %.

Eine Lösung von 2,7 g *Propiophenon* und 1,8 g Thiosemicarbazid in 40 ml warmem, 50 %igem Alkohol schied bis zum folgenden Tage 3,6 g Thiosemicarbazon als farblose Kristallkuchen vom Schmp. 114° – 115° aus. Bei der Umkristallisierung aus 10 ml gew. Alkohol wurden hieraus 3,0 g Thiosemicarbazon als farblose, flache Prismen erhalten, welche bei 115° – 117° ¹ schmolzen und 20,20, ber. 20,27, % N enthielten. — In der gleichen Weise, aber nach 3stündiger Erhitzung des Reaktionsgemisches auf dem Wasserbade, lieferten 2,7 g *Phenylazeton* mit 1,8 g Thiosemicarbazid 3,6 g Täfelchen vom Schmp. 121° – 125° und mit 19,33 % N. Beim Umkristallisieren aus 20 ml gew. Alkohol wurden hieraus 2,5 g flache Prismen erhalten, welche bei 125° – 130° schmolzen und 20,11, ber. 20,27, % N enthielten. Keines von diesen beiden Thiosemicarbazonen lieferte bei den Behandlungen mit Eisenchlorid definierbare Produkte.

Åkarp, Schweden, Mai 1955.

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¹ CHABRIER und CATTELAIN (13) fanden für dieses Thiosemicarbazon Schmp. 113° – 114° .

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Studies on the extraction of metal complexes. XIII*

The complex formation between U(IV) and acetylacetone.

By JAN RYDBERG and BRITTA RYDBERG

With 5 figures in the text

In spite of the importance of uranium compounds in atomic energy programmes, very few investigations of the complex formation of quadrivalent uranium have been reported. One reason for this is certainly to be found in the experimental difficulties encountered in work with U(IV). In dilute aqueous solutions of $\text{pH} > 0$, some U(IV) is always oxidized to U(VI), if no redox-buffer is present, even if great care is taken to prevent this oxidation. Since the presence of both U(VI) and a redox-buffer considerably complicates the investigations, most studies of U(IV) complexes have been of a qualitative nature [1, 2]. So far only four quantitative investigations have been reported.

In the spectrophotometric measurements on the U(IV)-OH⁻ and -Cl⁻ complexes by KRAUS and NELSON [3], corrections for changes in the spectra with time were made by extrapolating the data back to "time zero", i.e. the time when non-oxidized U(IV) was introduced into the system. This extrapolation includes a correction for oxidation of U(IV) to U(VI), in addition to the correction for "slow polymerization" mentioned by the authors.

In the potentiometric investigations on the U(IV) complexes with Cl⁻, Br⁻ and SCN⁻ by ÅHRLAND and LARSSON [4], and the U(IV) complexes with OH⁻ by HIETANEN [5], some U(VI) was also present. Since these authors had previously calculated formation constants for the U(VI)-Cl⁻, -Br⁻, -SCN⁻ [6, 7] and -OH⁻ [8] complexes, they could correct for the influence of U(VI) on the U(IV) complex system.

BETTS and LEIGH [9] used the thenoyltrifluoroacetone (TTA)-benzene-extraction technique [10] to study complexes between U(IV) and HSO₄⁻. On account of the large distribution coefficient of the U(TTA)₄ complex between benzene and strongly acidic aqueous solutions, the experiments could be carried out in 2M HClO₄, where it was assumed that the oxidation of U(IV) could be neglected. As will be shown later, this assumption does not hold in dilute U(IV) solutions. However, the presence of TTA and HSO₄⁻ stabilize the +4 valency state, so that the oxidation of U(IV) could be neglected in their case.

In all these cases one or more formation constants were reported (the results obtained by BETTS and LEIGH were later corrected by SULLIVAN and HINDMAN [11]). In addition to mononuclear complexes polynuclear complexes were reported

* Part XII: This journal, p. 101, 113.

for the hydroxyl complexes, which were found to be of the general "core and links" type suggested by SILLÉN [12].

This paper describes an investigation of the complex formation between U(IV) and acetylacetone (abbreviated to HAa). No study of this system in solutions has previously been reported, although the solid compound UAa₄ (needle-like olive green crystals, readily soluble in ether, alcohol and benzene) was prepared [13] more than 50 years ago. Since the stability constants of the complexes formed between U(VI) and HAa had been determined [14], corrections could be made for the presence of U(VI) in the U(IV) system.

As in previous investigations in this series [e.g. 14], the distribution ratio of the metal between the organic and aqueous solutions was measured as a function of pH and ligand concentration. From these measurements it was possible to calculate formation constants for the complexes UAa_n⁴⁻ⁿ, where 1 ≤ n ≤ 4, as well as the distribution constant for the uncharged UAa₄ between the organic and aqueous solvents.

Experimental

1. Chemicals

UO₃ of high purity, obtained from AB Atomenergi, was dissolved in p.a. HCl or HClO₄. Any solid residue was filtered off. The solution was then made 0.1M in UO₂²⁺ and electrolysed in a two compartment cell with a graphite anode and mercury cathode [15]. At a potential of 12 volts and a current of about 1 amp, the U(VI) in 200 ml of the solution was reduced to U(IV) in about an hour. The end of the reduction was indicated by the formation of red U(III). The U(III) was rapidly oxidized to U(IV) by short aeration.

The solution was stored in darkness in an ordinary bottle in an atmosphere of N₂. Samples were taken out from this bottle with pipettes; the bottle was then filled with new N₂ before it was closed again. The rate of oxidation of U(IV) in solutions prepared and stored in this manner does not exceed a few percent per month.

The organic solvent C₆H₆ was of analytical grade. It was washed several times with equal portions of H₂O before use.

Acetylacetone (Riedel de Haen, für Chromatographie) was purified as before [16]. The purity was checked by potentiometric titration.

All solutions used in the redox experiments were carefully freed from O₂ by prolonged purging with N₂ freed from O₂ according to MEYER and RONGE [17]. The distilled water, from which the solutions were prepared was freed from O₂ by bubbling N₂ through the boiling liquid, and then cooled and stored in an atmosphere of N₂. The argon gas used contained about 2 % N₂ and 0.0002 % O₂.

All other chemicals were of p.a. quality.

2. The determination of U(IV)

In order to find a suitable method for the determination of U(IV) in aqueous solutions containing U(VI), acetylacetone, C₆H₆, CHCl₃ and ClO₄⁻, a number of analytical procedures were tested. Since the concentration of U(IV) can be found either by direct analysis or from the difference between total uranium and U(VI), these procedures will include the determination of total uranium and U(VI).

For the *direct analysis of U(IV)* the following procedures were tested: (a) titration with

MnO_4^- [18, p. 68], (b) titration with $\text{Cr}_2\text{O}_7^{2-}$ [18, p. 68], (c) spectrophotometric determination at $655\text{ m}\mu$, (d) precipitation with IO_3^- [18, p. 20], and (e) precipitation with $\text{C}_2\text{O}_4^{2-}$ [18, pp. 24, 50]. In connection with the two last methods the total uranium in the precipitates was determined by method (j)–(l) (see below).

Of these methods (a) and (b) could not be used because of the simultaneous oxidation of acetylacetone, (c) was found to be too insensitive (molar extinction coefficient at the $655\text{ m}\mu$ peak in 1M HClO_4 is only 58) and unsuitable at $\text{pH} > 1$ (hydrolysed U(IV) species absorb in this wavelength region [3]), and method (d) did not give reproducible results. The only suitable method was the precipitation with oxalic acid, which was carried out in 1M H^+ at room temperature. The precipitates were allowed to stand overnight before they were separated from the solutions and analysed for uranium. It was found that in solutions of both U(IV) and U(VI) no reduction of U(VI) to U(IV) occurred during this time. At low concentrations of U(IV), Th^{4+} was found to be a suitable carrier. Approximately 0.1 % of the U(VI) in the solutions was carried down with the Th precipitate. Since this never exceeded 5 % of the amount of U(IV) in the precipitates, it has been neglected in the calculations.

For the direct determination of the concentration of U(VI), which was of especial interest in the redox experiments, three different methods were tested: (f) titration with Cr^{2+} [18, p. 62], (g) titration with Ti^{3+} [18, p. 64], and (h) extraction of U(VI) with HNO_3 and $\text{Mg}(\text{NO}_3)_2$ into diethyl ether [19], followed by a determination of total uranium in the ether phase.

In method (f) equilibrium was very slowly reached [cf. 4] and no sharp end point was obtained. (This may be due to the low concentration of Cr^{2+} in the reducing solution which was prepared according to STONE and BEESON [20].) Method (g) was found to be useful, if the titrations were carried out in 0.1M tartrate solutions at 65°C [21]. For low concentrations of U(VI), the extraction method (h) was found to give the best agreement with the direct analysis for U(IV), although the reproducibility was never better than $\pm 10\%$.

Depending on the amount of uranium and on other salts present, three different methods were used for the determination of total uranium: (j) ignition and weighing as U_3O_8 , (k) spectrophotometric determination of U(VI) with SCN^- [22] after the oxidation of all uranium to U(VI) with HClO_4 and HNO_3 , and (l) fluorimetric determination [23]. Though the accuracy of method (l) is in general not better than $\pm 10\%$, it has been extensively used in the experiments recorded in this paper because of its high sensitivity (down to 10^{-11} moles). The fluorophotometer used was constructed at AB Atomenergi [24]. However, when the sensitivity permitted, the spectrophotometric method (k) was preferred.

3. Some preliminary experiments on the hydrolysis and oxidation of U(IV)

Since it was found from spectrophotometric measurements that solutions of 0.1M U(IV) in 1M HCl or HClO_4 in an atmosphere of N_2 at 25°C were stable for quite a long time, it was hoped that the distribution experiments, which are recorded in the next section, could be carried out without any oxidation of U(IV). However, the distribution experiments were to be started with 10^{-3}M U(IV) and in the presence of acetylacetone, C_6H_6 or CHCl_3 , and at fairly low acidities ($\text{pH} \leq 6$). It was therefore necessary to obtain some information about the oxidation and hydrolysis of U(IV) in solutions of this kind before the distribution experiments could be started.

Alkali was therefore added to a 0.1M U(IV) solution in 1M HCl until a precipitate started to form ($\text{pH} \sim 3$); this was then dissolved in 1M HCl . It was then found that the molar extinction coefficient of the U(IV) peak at $655\text{ m}\mu$ had decreased by about 20 %, and that this decrease was constant for the next 20 hours. Since similar results were obtained in the presence of redox-buffers such as Ti^{3+} and Sn^{2+} , a 20 % oxidation of U(IV) to U(VI) was thought to be less probable

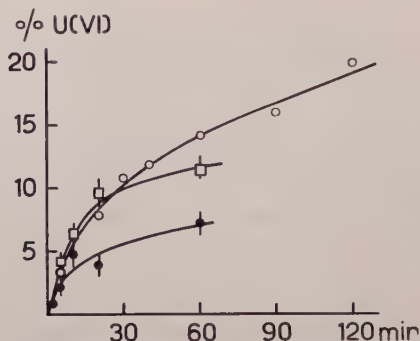


Fig. 1. The increase in concentration of U(VI) in a solution of U(IV) as a function of time at 25°C. Initial composition of solution is $10^{-3}M$ U(IV) in $0.01M$ $HClO_4$.

- N_2 bubbled through solution.
- A bubbled through solution.
- Solution kept below a layer of paraffin oil.

in these experiments. The decrease was therefore interpreted as due to an irreversible (within 20 hours) hydrolysis of U(IV).

In order to test if any oxidation took place in the absence of a redox-buffer and at lower concentrations of U(IV), a number of similar experiments was carried out. The precipitate was redissolved and the final solution analysed for U(VI). It was then found that a considerable fraction of U(IV) had been oxidized, and that this fraction increased with decreasing initial concentration of U(IV). Thus in one experiment with a solution of U(IV) of an initial concentration of $10^{-3}M$ about 30 % had been oxidized. This oxidation also occurred in strongly acid solutions. For instance, after a 5-fold dilution of a $0.1M$ solution of U(IV) in $1M$ $HClO_4$ with $1M$ $HClO_4$, the concentration of U(VI) increased by several percent. The amount of U(IV) oxidized in dilute solutions increases with time, as is shown in Fig. 1. Each point on the curves for N_2 and A in Fig. 1 is an average of 2-4 analyses. The oxidation in presence of argon cannot be explained by the small amount of O_2 in the gas.

From these experiments it could be concluded that no reliable predictions could be made regarding the relative amounts of U(IV) and U(VI) obtained at equilibrium in the distribution experiments. In every experiment the concentrations of U(IV) and U(VI) had to be determined separately.

4. The distribution experiment

As a consequence of the results described in the preceding sections, the distribution experiments were carried out in the following manner.

To the aqueous phase, which all the time was kept below an atmosphere of argon $HClO_4$, $NaClO_4$, U^{4+} and acetylacetone were added. The pH of the solution was then adjusted by careful addition of $NaOH$ or $NaHCO_3$. ($NaHCO_3$ was at first used instead of $NaOH$, since on the addition of $NaOH$ to the U(IV) solution, local excesses of alkali may produce irreversibly hydrolysed U(IV) species, which if possible should be avoided. It was believed that the carbonate complexes of uranium could be neglected, at least at low pH. Since it was later found that this was not the case, $NaOH$ was used in another set of experiments.) The amounts were so chosen that the final aqueous solution was $0.1M$ in $NaClO_4$. No visible precipitates were formed during this mixing. The total concentration of uranium in this solution will be denoted by $[U_t]_{aq}$. The benzene

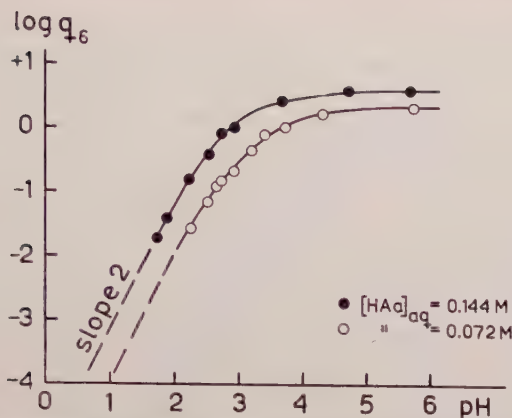


Fig. 2. The distribution ratio q_6 of U(VI) between benzene and 0.1M NaClO₄ at 25°C as a function of pH and concentration of acetylacetone in the aqueous phase.

phase was then added, and the two phases were shaken in the atmosphere of argon for 30 minutes at 25°C. This time was found to be quite sufficient for equilibrium to be established. In the solutions (pH > 2.1) to which NaOH had been added a precipitate appeared during the equilibration. No analyses were made of this precipitate, which amounted to $5 \pm 5\%$ of the total uranium present.

After the phases had been separated by centrifuging and the precipitate filtered away from the benzene phase, aliquots of the two phases were taken out, and analysed for total uranium, giving $[U_t]_{org}$ and $[U_t]_{aq}$. The aqueous phase was then analysed for U(IV) by method (e) above, giving $[U_4]_{aq}$, which in general constituted the minor fraction. As in previous papers of this series pH is given as $-\log[H^+]$, sometimes abbreviated to pH_c. It was determined with a valve potentiometer (pH-Meter, type 3i, Radiometer, Copenhagen) using glass and calomel electrodes. The pH-scale had been calibrated against known hydrogen ion concentrations in 0.1M NaClO₄.

Results

1. Calculation of the distribution curve

Only the total concentration of uranium in the organic phase, $[U_t]_{org}$, was determined. The U(IV) concentration, $[U_4]_{org}$, is obtained from this value and concentration of U(VI) in the organic phase, $[U_6]_{org}$. This latter concentration can be calculated if $[U_6]_{aq}$ and the distribution ratio q_6 of U(VI) between benzene and water are known. Since it has previously been shown [14] that this distribution ratio does not only depend on pH but also on the concentration of HAa in the aqueous phase, experimental curves had to be determined for the concentrations of HAa used here. These curves are shown in Fig. 2.

The distribution ratio of U(IV), q_4 , between the organic and aqueous phases can then be calculated from the relationship

$$q_4 = \frac{[U_t]_{org} - q_6 [U_6]_{aq}}{[U_4]_{aq}}$$

In Fig. 3 $\log q_4$ is given as a function of $\log[Aa^-]$, which has been calculated from the equation

$$-\log[Aa^-] = pk_a - pH - \log m + \log(v_{aq} + k_d \cdot v_{org} + v_{aq} k_a [H^+]^{-1}). \quad (1)$$

Table 1a. The calculation of the distribution ratio q_4 of U(IV) between benzene and aqueous solutions containing acetylacetone. The pH has been adjusted by the addition of NaOH. $[H\text{Aa}]_{\text{aq}} = 0.072M$, $[U_t]_{\text{aq}} = 0.0011M$, symbols $\circ$ in Fig. 3. All concentrations are given in $10^{-3}M$.

$-\log [Aa^-]$	pH	$[U_t]_{\text{aq}}$	$[U_4]_{\text{aq}}$	$[U_6]_{\text{aq}}$	q_6	$[U_6]_{\text{org}}$	$[U_t]_{\text{org}}$	$[U_4]_{\text{org}}$	$\log q_4$	$v_{\text{org}}/v_{\text{aq}}$
8.58	1.38	0.978	0.453	0.525	0.0005	~ 0	0.0106	0.011	-1.62	40/5
8.58	1.38	0.989	0.331	0.658	0.0005	~ 0	0.0102	0.010	-1.52	
8.48	1.48	0.917	0.370	0.547	0.0008	~ 0	0.0188	0.019	-1.29	
8.22	1.74	0.652	0.192	0.460	0.0033	0.002	0.0592	0.057	-0.53	
8.10	1.86	0.554	0.193	0.361	0.0059	0.002	0.0678	0.066	-0.47	
7.82	2.14	1.00	0.57	0.43	0.017	0.01	1.29	1.28	0.35	
7.62	2.34	0.89	0.37	0.52	0.038	0.02	2.41	2.39	0.81	
7.46	2.50	0.71	0.37	0.34	0.069	0.02	3.93	3.91	1.02	3/30
7.31	2.65	0.59	0.25	0.34	0.110	0.04	5.61	5.57	1.35	
7.14	2.82	0.42	0.16	0.26	0.182	0.05	6.73	6.68	1.62	
7.03	2.93	0.328	0.082	0.246	0.240	0.06	8.20	8.14	2.00	
6.83	3.13	0.229	0.053	0.176	0.40	0.07	9.42	9.35	2.25	
6.78	3.18	0.210	0.040	0.170	0.48	0.08	9.54	9.46	2.37	
6.56	3.40	0.163	0.017	0.146	0.73	0.11	9.46	9.35	2.74	
6.47	3.49	0.158	0.015	0.143	0.83	0.12	10.22	10.10	2.83	
6.43	3.53	0.120	0.018	0.102	0.87	0.09	9.60	9.51	2.72	
6.41	3.55	0.160	0.012	0.148	0.91	0.14	9.75	9.61	2.90	
6.10	3.86	0.092	0.0056	0.086	1.32	0.11	10.40	10.29	3.26	
6.08	3.88	0.100	0.0061	0.094	1.38	0.13	10.62	10.49	3.23	
5.93	4.03	0.080	0.0047	0.075	1.51	0.11	10.65	10.54	3.35	
5.70	4.26	0.063	0.0044	0.059	1.74	0.10	10.48	10.38	3.37	
5.63	4.33	0.088	0.0035	0.084	1.82	0.15	10.68	10.53	3.48	
5.54	4.42	0.081	0.0038	0.077	1.95	0.15	10.72	10.57	3.44	
5.25	4.71	0.074	0.0023	0.072	2.04	0.15	10.31	10.16	3.64	
5.21	4.75	0.074	0.0032	0.071	2.09	0.15	10.72	10.57	3.52	
5.01	4.95	0.080	0.0028	0.077	2.14	0.16	10.29	10.13	3.56	
4.45	5.51	0.080	0.0022	0.078	2.30	0.18	10.77	10.59	3.68	
4.12	5.84	0.063	0.0023	0.061	2.30	0.14	10.91	10.77	3.67	
3.89	6.07	0.049	0.0026	0.046	2.30	0.11	10.79	10.68	3.61	
3.88	6.08	0.063	0.0020	0.061	2.30	0.14	10.93	10.79	3.73	
3.84	6.12	0.046	0.0022	0.044	2.30	0.10	10.88	10.78	3.69	
3.80	6.16	0.059	0.0034	0.056	2.30	0.13	10.93	10.80	3.50	
3.56	6.40	0.076	0.0019	0.074	2.30	0.17	10.77	10.60	3.74	

The value of the constant pk_a in $0.1M$ NaClO_4 is 8.82 [25], k_a is 5.8 [16], m is the amount in moles of HAA added, and v_{aq} and v_{org} the equilibrium volumes of the aqueous and organic phases respectively.

The experimental values and the calculation of q_4 are given in Tables 1 and 2.

2. Analysis of the distribution data

It is seen from the two sets of points in Fig. 3 that the values obtained in the presence of carbonate are definitely lower than the other values. On account of the analytical methods used, the formation of carbonate complexes of U(VI) as well as of U(IV) will lead to lower values of q_4 , if it is assumed that the carbonate complexes remain in the aqueous phase. These carbonate complexes are already formed in appreciable amounts even at values of $\log[Aa^-] \approx -8$, which in our solutions

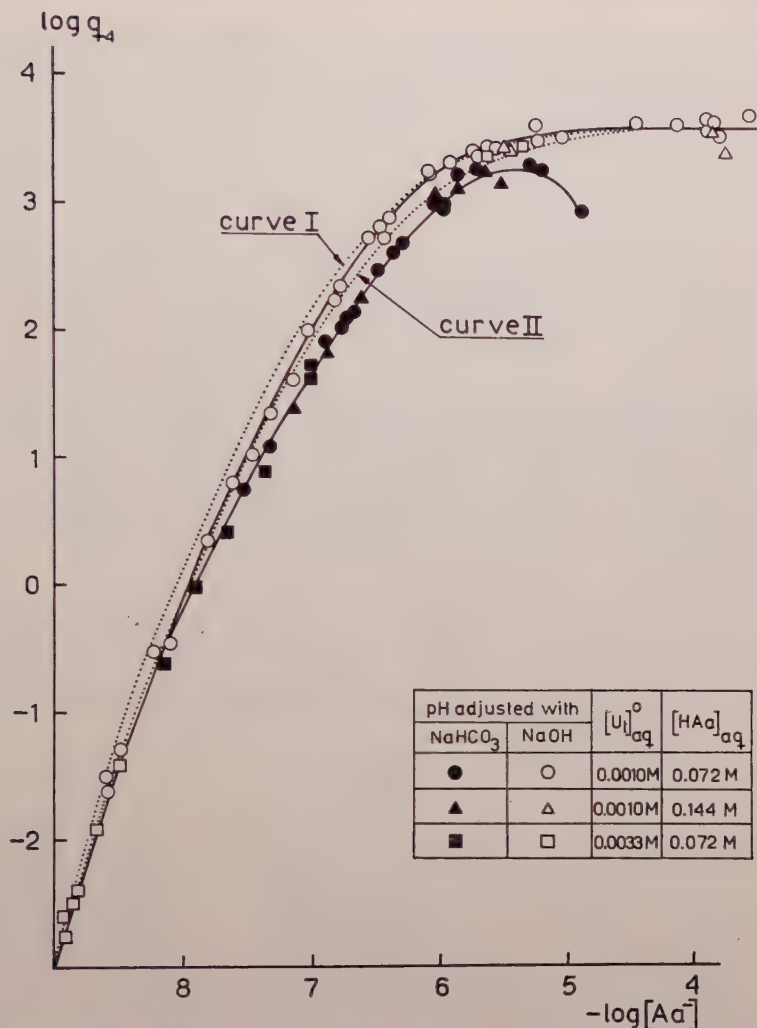


Fig. 3. The distribution ratio q_4 of U(IV) between benzene and 0.1M $NaClO_4$ at 25°C as a function of the concentration of acetylacetonate ion in the aqueous phase. $[U]_{aq}^o$ is the total concentration of uranium originally present in the aqueous phase; $[U_4]_{aq}^o \geq 10[U_6]_{aq}^o$.

correspond to $pH \approx 2$. Since the concentration of HCO_3^- at this pH is very small, the formation constants for the carbonate complexes must be very great. However, since the concentration of carbonate ions in these solutions is not known (CO_2 was partly bubbled out of these solutions during the time of mixing which usually was < 1 minute), it is not possible to make any quantitative estimates of the size of the formation constants of the carbonate complexes. On account of the carbonate complexing the curve through the black points in Fig. 3 has not been used in the following calculations.

In a previous paper [26] it was pointed out that in order to use a plot of the type $\log q$ versus $-\log[Aa^-]$ to calculate formation constants, one had to prove that no

Table 1b. The calculation of the distribution ratio q_4 of U(IV) between benzene and aqueous solutions containing acetylacetone. The pH has been adjusted by the addition of NaOH. $[HAA]_{aq} = 0.144M$, $[U_t]_{aq} = 0.0011M$, $v_{org} = 3$ ml, $v_{aq} = 30$ ml, symbols Δ in Fig. 3. All concentrations are given in $10^{-3}M$.

$-\log [Aa^-]$	pH	$[U_t]_{aq}$	$[U_4]_{aq}$	$[U_6]_{aq}$	q_6	$[U_6]_{org}$	$[U_t]_{org}$	$[U_4]_{org}$	$\log q_4$
5.52	4.14	0.046	0.0039	0.042	3.80	0.16	11.25	11.09	3.45
5.46	4.20	0.051	0.0043	0.047	3.80	0.18	11.40	11.22	3.42
3.86	5.80	0.052	0.0028	0.049	4.35	0.21	11.55	11.34	3.61
3.75	5.91	0.051	0.0039	0.047	4.35	0.21	11.08	10.87	3.44

polynuclear complexes and no complexes with excess of OH^- or undissociated HAA were formed. The first criterion requires that q should be independent of $[U]$, and the second that q should be independent of $[HAA]$ at constant $[Aa^-]$.

Two different total initial concentrations of uranium in the aqueous phase were tested, $10^{-3}M$ and $3.3 \times 10^{-3}M$. It is seen from Fig. 3 that the points for the two uranium concentrations fall on the same line. It is therefore concluded that polynuclear complexes can be neglected.

Table 1c. The calculation of the distribution ratio q_4 of U(IV) between benzene and aqueous solutions containing acetylacetone. The pH has been adjusted by the addition of NaOH. $[HAA]_{aq} = 0.072$, $[U_t]_{aq} = 0.0033-0.0036M$, $v_{org} = v_{aq} = 15$ ml, symbols $\square$ in Fig. 3. All concentrations are given in $10^{-3}M$.

$-\log [Aa^-]$	pH	$[U_t]_{aq}$	$[U_4]_{aq}$	$[U_6]_{aq}$	q_6	$[U_6]_{org}$	$[U_t]_{org}$	$[U_4]_{org}$	$\log q_4$
8.94	1.02	3.74	3.52	0.220	0.0001	~ 0	0.0087	0.0087	-2.61
8.90	1.06	3.74	3.50	0.236	0.0001	~ 0	0.0059	0.0059	-2.77
8.84	1.12	3.58	3.37	0.206	0.0001	~ 0	0.1050	0.0105	-2.51
8.79	1.17	3.60	3.26	0.342	0.0002	~ 0	0.0130	0.0130	-2.40
8.66	1.30	3.53	3.25	0.276	0.0004	~ 0	0.0390	0.0390	-1.92
8.48	1.48	3.26	2.81	0.452	0.0010	~ 0	0.1077	0.1077	-1.42
5.63	4.33	0.010	0.0015	0.0085	1.90	0.02	3.43	3.41	3.36
5.38	4.58	0.014	0.0013	0.0130	2.09	0.03	3.61	3.58	3.44

Two different concentrations of HAA, $0.144M$ and $0.072M$, have also been used in the aqueous phase. If complexes with excess of OH^- or HAA were formed this would lead to a change (usually an increase) in q_4 with increasing $[HAA]$. As is seen from Fig. 3 the points with the higher concentration of HAA agree with the other points. Complexes with OH^- and HAA will therefore be neglected.

3. Calculation of formation constants

Since the distribution ratio q_4 is found to be a function of $[Aa^-]$ only it can be shown [26] that only complexes of type UAa_n^{4-n} have to be considered. The distribution ratio should then vary with $[Aa^-]$ according to

$$q_4 = \frac{\lambda_4 \kappa_4 [Aa^-]^4}{\sum \kappa_n [Aa^-]^n} \quad (2)$$

Table 2a. Calculation of the distribution ratio q_4 of U(IV) between benzene and aqueous solutions containing acetylacetone. The pH has been adjusted by the addition of NaHCO_3 . $[\text{HAA}]_{\text{aq}} = 0.072M$, $[\text{U}]_{\text{aq}}^\circ = 0.0011\text{--}0.00099M$, $v_{\text{org}} = 3\text{ ml}$, $v_{\text{aq}} = 30\text{ ml}$, symbols ● in Fig. 3. All concentrations are given in $10^{-3}M$.

$-\log [\text{Aa}^-]$	pH	$[\text{U}_t]_{\text{aq}}$	$[\text{U}_4]_{\text{aq}}$	$[\text{U}_6]_{\text{aq}}$	q_6	$[\text{U}_6]_{\text{org}}$	$[\text{U}_t]_{\text{org}}$	$[\text{U}_4]_{\text{org}}$	$\log q_4$
7.52	2.44	0.738	0.570	0.168	0.055	0.009	3.23	3.22	0.75
7.33	2.63	0.528	0.432	0.096	0.102	0.010	5.36	5.35	1.09
6.90	3.06	0.221	0.093	0.128	0.35	0.045	8.04	8.00	1.93
6.77	3.19	0.134	0.093	0.041	0.48	0.020	9.79	9.77	2.02
6.73	3.23	0.183	0.070	0.113	0.53	0.061	8.70	8.64	2.09
6.68	3.28	0.102	0.073	0.029	0.58	0.017	10.13	10.11	2.14
6.47	3.49	0.074	0.034	0.040	0.83	0.033	10.38	10.35	2.48
6.37	3.59	0.054	0.026	0.028	1.00	0.028	10.98	10.95	2.62
6.29	3.67	0.210	0.024	0.186	1.10	0.21	11.63	11.42	2.68
6.07	3.89	0.052	0.011	0.041	1.38	0.06	11.78	11.72	3.03
5.97	3.99	0.063	0.012	0.051	1.48	0.08	11.45	11.37	2.98
5.95	4.01	0.040	0.013	0.027	1.52	0.04	10.95	10.91	2.92
5.87	4.09	0.024	0.0063	0.018	1.58	0.03	11.48	11.45	3.26
5.72	4.24	0.036	0.0055	0.030	1.70	0.05	11.70	11.65	3.33
5.29	4.67	0.045	0.0050	0.040	1.99	0.08	11.54	11.46	3.36
5.21	4.75	0.027	0.0059	0.021	2.09	0.04	11.30	11.26	3.28
4.87	5.09	0.027	0.012	0.015	2.19	0.03	10.82	10.79	2.95

where κ_n is the overall complexity constant of UAa_n^{4-n} defined by

$$\kappa_n = \frac{[\text{UAa}_n^{4-n}]}{[\text{U}^{4+}][\text{Aa}^-]^n}, \tag{3}$$

and λ_4 is the distribution constant of uncharged UAa_4 between the two phases

$$\lambda_4 = \frac{[\text{UAa}_4]_{\text{org}}}{[\text{UAa}_4]_{\text{aq}}}. \tag{4}$$

Table 2b. Calculation of the distribution ratio q_4 of U(IV) between benzene and aqueous solutions containing acetylacetone. The pH has been adjusted by the addition of NaHCO_3 . $[\text{HAA}]_{\text{aq}} = 0.144M$, $[\text{U}]_{\text{aq}}^\circ = 0.0011M$, $v_{\text{org}} = 3\text{ ml}$, $v_{\text{aq}} = 30\text{ ml}$, symbols ▲ in Fig. 3. All concentrations are given in $10^{-3}M$.

$-\log [\text{Aa}^-]$	pH	$[\text{U}_t]_{\text{aq}}$	$[\text{U}_4]_{\text{aq}}$	$[\text{U}_6]_{\text{aq}}$	q_6	$[\text{U}_6]_{\text{org}}$	$[\text{U}_t]_{\text{org}}$	$[\text{U}_4]_{\text{org}}$	$\log q_4$
7.13	2.53	0.348	0.313	0.035	0.48	0.017	7.89	7.87	1.40
6.88	2.78	0.168	0.143	0.025	0.87	0.022	9.85	9.83	1.84
6.61	3.05	0.090	0.058	0.032	1.45	0.046	10.87	10.82	2.27
6.03	3.63	0.042	0.0092	0.033	2.63	0.09	11.40	11.31	3.09
5.86	3.80	0.034	0.0076	0.026	3.02	0.08	10.68	10.60	3.14
5.69	3.97	0.029	0.0053	0.024	3.31	0.08	11.43	11.35	3.33
5.51	4.15	0.046	0.0073	0.039	3.62	0.14	11.27	11.13	3.18

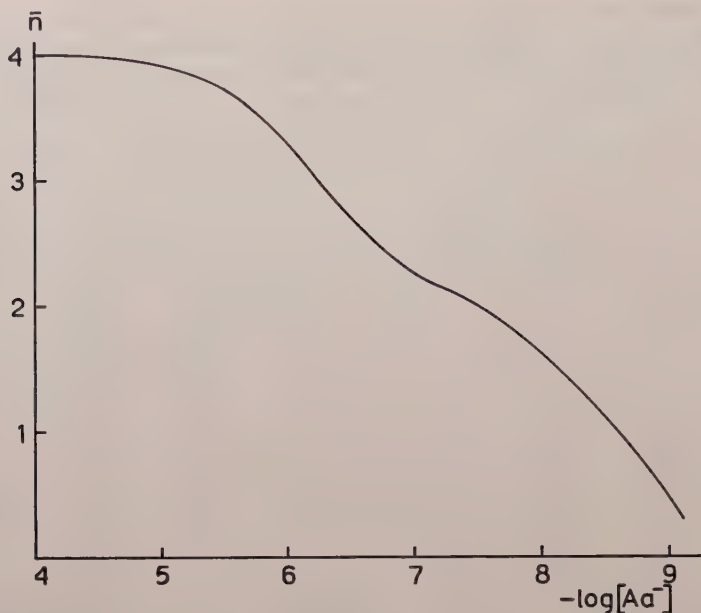


Fig. 4. The average number $\bar{n}$ of acetylacetonate ligands per U(IV) atom as a function of the concentration of free acetylacetonate ion.

The relation between κ_n and the successive formation constants k ($n \geq 1$), defined by

$$k_n = \frac{[UAa_n^{4-n}]}{[UAa_{n-1}^{5-n}][Aa^-]} \quad (5)$$

is given by

$$\kappa_n = \prod k_n. \quad (6)$$

In the first attempt to obtain the overall complexity constants the "two parameter method" [27] was used. This method is based on the assumption that the ratio between two successive formation constants, k_n/k_{n+1} , is constant. When this is the case, the complexity constants can very rapidly be calculated from the value of the intercept of the two asymptotes with slope -4 and 0 of the distribution curve, and the vertical distance between this intercept and the distribution curve. From the two following sets of κ_n values, which were obtained by this method,

$\log \kappa_1 = 8.94$	$\log \kappa_1 = 9.15$
$\log \kappa_2 = 16.88$	$\log \kappa_2 = 17.16$
$\log \kappa_3 = 23.82$	$\log \kappa_3 = 24.03$
$\log \kappa_4 = 29.76$	$\log \kappa_4 = 29.76$
(Curve I)	(Curve II)

and $\log \lambda_4 = 3.62$, two curves were calculated (see Fig. 3) which fit the experimental data well at high q -values (Curve I) or well at low q -values (Curve II). With other values of κ_n and λ_4 one can obtain curves in between these two, which as an average over the whole q -range will fit the experimental data better than Curves I and II. However, these curves will fit the data less good than Curve I at high q -values, and less good than Curve II at low q -values.

Table 2c. Calculation of the distribution ratio q_4 of U(IV) between benzene and aqueous solutions containing acetylacetone. The pH has been adjusted by the addition of NaHCO_3 . $[\text{HAA}]_{\text{aq}} = 0.072M$, $[\text{U}_t]_{\text{aq}} = 0.0033\text{--}0.0036M$, $v_{\text{org}} = v_{\text{aq}} = 15\text{ ml}$, symbols ■ in Fig. 3. All concentrations are given in $10^{-3}M$.

$-\log [\text{Aa}^-]$	pH	$[\text{U}_t]_{\text{aq}}$	$[\text{U}_4]_{\text{aq}}$	$[\text{U}_6]_{\text{aq}}$	q_6	$[\text{U}_6]_{\text{org}}$	$[\text{U}_t]_{\text{org}}$	$[\text{U}_4]_{\text{org}}$	$\log q_4$
8.14	1.82	2.83	2.33	0.503	0.007	0.004	0.545	0.541	-0.63
7.89	2.07	1.920	1.525	0.395	0.013	0.005	1.514	1.51	0.00
7.65	2.31	1.218	0.794	0.424	0.033	0.014	2.18	2.17	0.44
7.36	2.60	0.743	0.341	0.402	0.098	0.039	2.74	2.70	0.90
7.03	2.93	0.190	0.065	0.125	0.24	0.030	3.63	3.60	1.74
7.02	2.94	0.178	0.072	0.106	0.25	0.026	3.25	3.22	1.65

Several more general methods can be used to obtain the constants in eq. (2) from the experimental data in Fig. 3. In this paper "the ligand number method" [cf. 11] will be used.

It has been shown [28] that the slope of the distribution curve is proportional to the ligand number $\bar{n}$, i.e. the average number of ligands per central atom. Since the

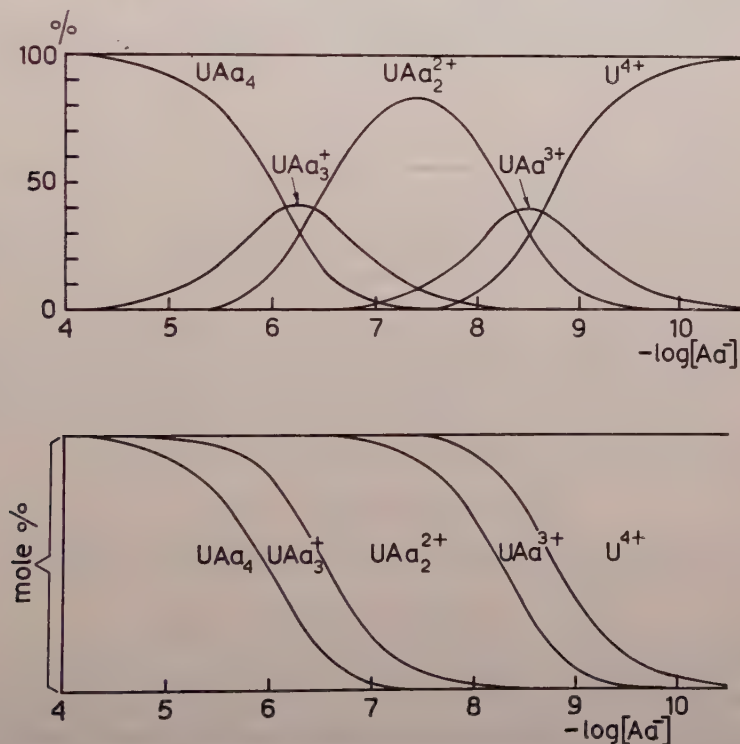


Fig. 5. The percentage of the different UAa_n^{4-n} complexes as a function of the concentration of free acetylacetonate ion. In the upper figure, the percentage of a complex is the height of the curve over the zero line. In the lower figure, the percentage is the length of the vertical line that falls in the area of that complex.

Table 3. The equilibrium constants obtained for the UAa_n^{4-n} complexes in 0.1M NaClO_4 at 25°C; κ_n is defined by eq. (3), k_n by eq. (5), and λ_4 by eq. (4).

$\log \kappa_1 = 8.6 \pm 0.2$	$\kappa_1 = 4.0 \times 10^8$	$\log k_1 = 8.6 \pm 0.2$	$k_1 = 4.0 \times 10^8$
$\log \kappa_2 = 17.0 \pm 0.2$	$\kappa_2 = 1.0 \times 10^{17}$	$\log k_2 = 8.4 \pm 0.4$	$k_2 = 2.5 \times 10^8$
$\log \kappa_3 = 23.4 \pm 0.1$	$\kappa_3 = 2.5 \times 10^{23}$	$\log k_3 = 6.4 \pm 0.3$	$k_3 = 2.5 \times 10^6$
$\log \kappa_4 = 29.5 \pm 0.1$	$\kappa_4 = 3.2 \times 10^{29}$	$\log k_4 = 6.1 \pm 0.2$	$k_4 = 1.3 \times 10^6$
	$\log \lambda_4 = 3.62 \pm 0.05$	$\lambda_4 = 4.2 \times 10^3$	

maximum coordination number of U(IV) towards acetylacetone is assumed to be 4, and the curve in Fig. 3 has a slope between -4 and 0 , this relation can be given as

$$\bar{n} = 4 - d(\log q_4)/d(\log [\text{Aa}^-]).$$

$\bar{n}$ can be obtained as a function of $\log [\text{Aa}^-]$ by graphical differentiation of a curve through the open points in Fig. 3. The result of such a differentiation is given in Fig. 4.

The connection between $\bar{n}$, κ_n and $[\text{Aa}^-]$ has been given by BJERRUM [29]:

$$\bar{n} + (\bar{n} - 1)\kappa_1[\text{Aa}^-] + (\bar{n} - 2)\kappa_2[\text{Aa}^-]^2 + (\bar{n} - 3)\kappa_3[\text{Aa}^-]^3 + (\bar{n} - 4)\kappa_4[\text{Aa}^-]^4 = 0.$$

By choosing four corresponding values of $\bar{n}$ and $[\text{Aa}^-]$ four equations are obtained, which can be solved for κ_n by the use of determinants. By choosing the values of $[\text{Aa}^-]$ at $\bar{n} = 0.5, 1.5, 2.5$, and 3.5 preliminary values of κ_n were calculated. These values were refined by successive approximations so that the distribution curve calculated from the value of the constants and eq. (2) fitted the experimental points. The best curve is drawn through these points in Figure 3. The κ_n and λ_4 values obtained are given in Table 3, together with the corresponding k_n values which have been calculated with eq. 6.

The distribution ratio λ_4 of the uncharged UAa_4 complex was obtained from the height of the plateau in Fig. 3, where q_4 is independent of $[\text{Aa}^-]$. It can be shown [26] that at this plateau $\log q_4 = \log \lambda_4$. Thus a value of 3.62 is obtained for $\log \lambda_4$, or $\lambda_4 = 4.2 \times 10^3$.

The fraction of each complex present at different concentrations of Aa^- has been calculated with the constants given in Table 3. The result is shown in Fig. 5. From this Figure the complex UAa_2^{2+} appears to be considerably more stable than UAa^{3+} and UAa_3^+ . Since the errors in the stability constants are fairly large (see Table 3) this higher stability cannot be regarded as conclusively proved.

The possible presence of U(V)

NELSON and KRAUS [30] have given the thermodynamic equilibrium constant 1.7×10^6 for the reaction



From this it follows that at $\text{pH} > 2$ the main uranium species should be U(V). Since much higher values of pH have been used here, one would believe that U(V) is the dominating valency state of the uranium species in our solutions. However, the following considerations will show that the presence of U(V) can be neglected in our case.

In general, Np(V) and Pu(V) form very weak complexes or none at all with anions, which give strong complexes with the $+4$ and $+6$ valency states of neptunium and

plutonium [31, 32]. Since U is chemically very similar to Pu and Np, when in the same valency state, the presence of complexing agents such as acetylacetone will certainly stabilize the U(IV) and U(VI) valency states at the expense of U(V). By introducing approximate equilibrium values for the U(IV) and U(VI) acetylacetone complexes, it can be found that the ratio $[U(V)]/[U_{\text{tot}}] < 10^{-14}$ exists in the concentration ranges measured, if no complexes between U(V) and acetylacetone are formed. In order to reach a ratio of the order of 1, U(V) would have to form much stronger complexes with acetylacetone than U(IV) and U(VI). It seems very improbable that it should be so.

Precipitation with oxalic acid is used to determine U(IV) in the aqueous solutions. The U(VI) oxalate is not precipitated under the conditions used. Since the U(V) oxalate should be still more soluble, it is assumed that the only uranium in the precipitate is U(IV). This assumption is strongly supported [cf. 33] by the fact that no Np(V) oxalate is precipitated from solutions $10^{-3}M$ in Np(V), $0.1M$ in $HC_2O_4^-$ at pH 3.2 [34]. Under the same conditions both Np(IV) and Np(VI) as well as U(IV) and U(VI) are precipitated. If some U(V) was present together with the U(VI) in the aqueous phase, the values for $[U_6]_{\text{aq}}$ and consequently also for $[U_6]_{\text{org}}$ would be lower than assumed in the calculations. However, it can be seen from Tables 1 and 2 that such a correction would have little influence on $[U_4]_{\text{org}} = [U_t]_{\text{org}} - [U_6]_{\text{org}}$.

From these considerations it is concluded that no errors due to the neglecting of U(V) have been made in the distribution or equilibrium constants for U(IV).

Summary

The complex formation between U(IV) and acetylacetone (HAa) has been studied by means of an extraction technique described previously [26, 28]. On account of the oxidation of U(IV) several experimental difficulties had to be solved before reliable values on the distribution ratio q_4 of U(IV) between the benzene and the aqueous phases could be obtained. From the distribution curve, $\log q_4 = f(\log [Aa^-])$, it could be concluded that only complexes of the type UAa_n^{4-n} need be considered. Equilibrium values for the formation of these complexes in $0.1M$ $NaClO_4$ at $25^\circ C$, as well as the distribution constant of UAa_4 between the organic and aqueous phases, could then be calculated and are given in Table 3.

The authors are indebted to the head of this institute, Prof. G. LJUNGGREN, for his kind interest in this work.

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Studies on the extraction of metal complexes. XIV

The separation of metal acetylacetonates

By JAN RYDBERG

With 3 figures in the text

Many metals can be extracted from aqueous solutions into inert organic solvents, such as CHCl_3 or C_6H_6 , in the presence of certain organic complexing reagents. Since different metals are generally extracted to a different extent under the same conditions, they may often be separated from each other by this method [e.g. 1, 2, 3]. Only a few organic reagents have been extensively used for this purpose, e.g. oxine, cupferron, dithizone and thenoyltrifluoroacetone ("TTA"), which all form chelate compounds with the extractable metal [4]. The object of the present paper is to show under what conditions a number of metals can be separated from each other by solvent extraction in the presence of acetylacetone, which is one of the simplest and most inexpensive of all chelating agents.

Studies on the extraction of acetylacetonates

STENE [5] was able to extract Fe (III), Cu(II), Al, Be and Ce from an aqueous acetate solution of pH 4.5–7.5 into CCl_4 containing 5 % (by volume) of acetylacetone. Under the same conditions the following metals were not extracted: Ca, Ba, Sr, Mg, Zn, Cd, Mn, Co, Ni, Pb, Hg, Ti, Cr, alkali metals, U(VI), Fe(II) and Cu(I). In some cases, e.g. in the extraction of Cu(II), CHCl_3 was found to extract more completely and rapidly than CCl_4 .

ABRAHAMCZIK [6] reports that Fe, Al, Mn, Ti, Cu, V and U can be extracted from aqueous NaHCO_3 solutions into CCl_4 containing 20 % acetylacetone.

STENE and ABRAHAMCZIK give few details about the conditions for the extractions, and mention the valency states of only few of the metals investigated by them.

In addition to these two investigations on the use of acetylacetone for the separation of metals in solvent extraction, a number of distribution or extraction curves for metal acetylacetonates have been published. Such curves can be used as a basis for devising separation processes.

STEINBACH and FREISER [7, 8] have given curves for the extraction of some metals from aqueous solutions of varying pH into pure acetylacetone.¹ They found that

¹ At room temperature and pH < 9 acetylacetone and H_2O are only partially miscible: H_2O can dissolve about 15 % (by weight) of acetylacetone, and acetylacetone can dissolve about 5 % of H_2O [8, 9].

50 % of the metals was extracted at about the following pH: Fe(III) 0, Be 0.7, Cu 1.1, Ga 1.1, Al 1.6, In 1.6 and Zn 5.1. All the metals could be extracted to ≥ 90 %, in one step except for Zn (about 60 %).

HARVEY *et al.* [10] found that the distribution ratio of Pu(IV) acetylacetonate between benzene and water, increased from about 0.5 at pH 1 to about 80 at pH > 5. No information is given about the ligand concentration.

In the author's studies on the formation and extraction of metal acetylacetonates, the distribution of the following metals between H_2O and the organic solvents $CHCl_3$, C_6H_6 and $C_4H_9COCH_3$ (methyl isobutyl ketone, "hexone") have previously been investigated: Th [11, 12, 13], U(VI) [13], U(IV) [14], Pu(IV) [15], La and Sm [16]. The conditions under which these metals have been studied are summarized in Tables 1-3. In addition to these metals, the extraction of K, Sr, Hf, U(IV) and a mixture of fission products (FP) is reported in this paper.

The extraction of metal complexes from aqueous into organic solvents

When a metal (Me) is extracted from an aqueous solution into an organic solvent in the presence of an organic complexing reagent (HA), the distribution ratio (q) of the metal between the organic and aqueous phases will be a function of several variables, such as pH, HA concentration, and metal concentration:

$$q = [Me]_{org}/[Me]_{aq} = f([Me], [HA], [H^+], \text{etc.});$$

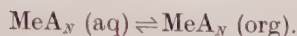
square brackets are used to indicate concentrations.

A plot of the distribution ratio q (or percentage extraction E , where $E = 100 q/(1 + q)$ for equal phase volumes) as a function of one of these variables will be referred to as a distribution (or extraction) curve. Since the exact influence of these variables on q has been discussed in detail elsewhere [17, 18], it will only be briefly outlined here.

If adsorption, and other phenomena which may occur at very low concentrations of metal are neglected, any variation of the distribution ratio with the concentration of metal probably indicates the formation of polynuclear complexes. Complexes of this type do not appear to be formed at metal concentrations $\leq 10^{-3}M$, except when the metal ion is strongly hydrolysed.

It should be noted that in a series of extraction experiments the metal concentration in each phase is not constant, but varies with the distribution ratio. At low values of q the metal concentration in the aqueous phase ($[Me]_{aq}$) may be high and polynuclear complexes may be formed. With increasing distribution ratios, $[Me]_{aq}$ decreases, perhaps to such an extent that the aqueous phase will only contain mononuclear complexes of the metal. On the other hand, polynuclear complexes may now be formed in the organic phase. The metal concentration in the distribution experiments listed in Tables 1-6 is therefore given as the original concentration in the aqueous phase ($[Me]_{aq}^0$), before any partition of the metal between the two phases has occurred.

It can be shown [e.g. 17] that the distribution ratio q or percentage extraction E of a metal is most conveniently plotted against the free ligand concentration $[A^-]$, since for many metals q and E are independent of $[HA]$ and $[H^+]$ at constant $[HA]$ $[H^+]^{-1}$ ($[A^-] = k_a[HA][H^+]^{-1}$, where k_a is the acid dissociation constant of the ligand) in a wide range of pH and ligand concentration. When this is found to be the case it can be assumed that the complexes formed are of the general form MeA_n , and the extracted complex is MeA_n :



If $N > 1$, the formation of the uncharged complex MeA_N involves the stepwise formation of the intermediate complexes MeA_1^{N-1} , MeA_2^{N-2} , etc.

It is sometimes found that q or E is not a function of $[\text{A}^-]$ only; at constant $[\text{A}^-]$ q may vary with $[\text{HA}]$. It can be shown [17] that this may be due to one or both of two different processes: (a) a mixed complex with an excess of OH (over HA) is formed, e.g. $\text{MeA}_n(\text{OH})_p$, (b) a mixed complex with an excess of HA (over OH) is formed, as e.g. $\text{MeA}_n(\text{HA})_r$. In such cases it is not sufficient to plot q or E against $[\text{A}^-]$; the HA concentration or pH must also be given.

On the addition of HA to a metal complex containing OH-groups, the equilibrium will shift towards complexes with less OH-groups, e.g.



Since these latter complexes are practically always more easily extracted than the mixed hydroxyl complexes, q or E will in general increase with $[\text{HA}]$, until all Me is bound in the final complex $\text{MeA}_N(\text{HA})_R$ ($R \geq 0$).

Table 1. The concentration ranges studied in the extraction of the metals Me shown in Fig. 1 from 0.1M NaClO_4 solutions into CHCl_3 at 25°C. All concentrations are in moles/l.

Me	$[\text{Me}]_{\text{aq}}^0$	$[\text{HAa}]_{\text{aq}}$	$[\text{HAa}]_{\text{org}}$	$E([\text{HAa}])_{\text{pAa}}$	Ref.
K	1.0×10^{-1}	0.020	0.48	no extraction	Table 8
Sr	1.0×10^{-3}	0.040	0.95	no extraction	Table 4
La	2.5×10^{-6}	≤ 0.002	≤ 0.05	increases	[16]
Sm	$(0.2 - 1.8) \times 10^{-6}$	≤ 0.002	≤ 0.05	increases	[16]
Hf	2.6×10^{-5}	≤ 0.002	≤ 0.05	increases	Table 5a
Th	1.8×10^{-6}	0.002 - 0.0004	0.05 - 0.01	independent	[12]
U(IV)	1.0×10^{-3}	0.02	0.5	independent*	Table 7
Pu(IV)	3.0×10^{-6}	0.04	1.0	independent*	[15]
U(VI)	1.0×10^{-3}	0.043 - 0.0004	1.0 - 0.010	increases	[13]

* Expected (not investigated).

Table 2. The concentration ranges studied in the extraction of the metals Me shown in Fig. 2 from 0.1M NaClO_4 solutions into C_6H_6 at 25°C. All concentrations are in moles/l.

Me	$[\text{Me}]_{\text{aq}}^0$	$[\text{HAa}]_{\text{aq}}$	$[\text{HAa}]_{\text{org}}$	$E([\text{HAa}])_{\text{pAa}}$	Ref.
Sm	4.7×10^{-6}	≤ 0.008	≤ 0.05	increases	[16]
Th*	carrier free	0.1 - 0.0007	0.7 - 0.004	independent	[11]
U(IV)	$(0.3 - 1.0) \times 10^{-3}$	0.14 - 0.07	0.86 - 0.43	independent	[14]
Pu(IV)	$(3 - 6) \times 10^{-6}$	0.14 - 0.007	0.86 - 0.04	independent	[15]
U(VI)	1.0×10^{-3}	0.14 - 0.02	0.86 - 0.13	increases	[13, 14]
FP	$< 10^{-8}$	0.10	0.60	increases ?	Table 6

* Aqueous phase only 0.01M in NaClO_4 .

Table 3. The concentration ranges studied in the extraction of the metals Me shown in Fig. 3 from 0.1M NaClO₄ solutions into hexone (metyl isobutyl ketone) at 25°C. All concentrations are in moles/l.

Me	[Me] _{aq} ⁰	[HAa] _{aq}	[HAa] _{org}	$E([HAa])_{pAa}$	Ref.
La	$(0.1 - 7) \times 10^{-6}$	0.17 - 0.008	1.0 - 0.05	independent	[16]
Hf	2.6×10^{-8}	≤ 0.007	≤ 0.04	increases	Table 5b
Th	2×10^{-6}	0.08 - 0.025	0.5 - 0.15	independent	[13]
U(VI)	1.0×10^{-8}	0.021	0.12	increases*	[13]

* Expected (not investigated).

The general trend of the percentage of the metal extracted as a function of [HA] at constant [A⁻] is given in Tables 1-3 (column $E([HAa])_{pAa}$, where HA = HAa = acetylacetone) for the ranges of A⁻ and HA concentrations investigated.

Experimental

The discussion of the extraction curves for Th, U(IV), U(VI), Pu(IV), La and Sm is based on experiments reported elsewhere [11-16], while the discussion of the extraction curves for Hf, U(IV) in CHCl₃, Sr, K and a mixture of fission products (FP) is based on the experiments described below.

Chemicals

HfO₂ was obtained as a pile irradiated unit from A.E.R.E., Harwell, England. The oxide was of analytical purity. It was not used until several months after the end of the irradiation; no significant deviation from its expected disintegration curve could then be found.

The oxide was treated with HCl and a little HF for a few hours at 80°C. The solution was then centrifuged and decanted. Hafnium hydroxide was precipitated by the addition of alkali, and the precipitate washed and dissolved in HClO₄. The precipitation procedure was repeated once and the hydroxide again dissolved in HClO₄. This solution was then used for the distribution experiments.

The U(IV) solutions were prepared as described in a previous paper [14].

Sr⁸⁹, in the form of SrCO₃, was obtained as a pile irradiated unit from A.E.R.E., Harwell. The Sr(ClO₄)₂ solutions were prepared by dissolving the carbonate in p.a. HClO₄.

The NaClO₄ and KClO₄ solutions were prepared by mixing solutions containing equivalent amounts of p.a. HClO₄ and NaOH or KOH, respectively.

The fission product (FP) mixture was prepared by dissolving neutron irradiated uranium metal in p.a. HNO₃, heating the solution and then withdrawing a certain amount, which was diluted with p.a. HClO₄. For each experiment a fraction of this solution was further diluted with ClO₄⁻ to make the aqueous solution in the distribution experiments 0.1M in ClO₄⁻, < 10⁻⁴M in NO₃⁻, < 5 × 10⁻⁵M in [U], and < 10⁻³M in Pu and FP. The uranium had been irradiated with slow neutrons at a low flux for about a year, and had cooled for approximately the same time.

The CHCl₃ and C₆H₆, of p.a. quality, were washed several times with water before use. Hexone of technical grade was washed several times with acid, alkali and finally water in order to remove water soluble impurities. The water used was distilled once.

Acetylacetone, which was generally of p.a. quality, was purified as described previously [19].

Procedure

The extraction experiments were carried out in the same way as previously described [12, 14]. The aqueous phase (to which had been added the metal ions, acetylacetone, NaOH and HClO_4 to give a suitable pH and an ionic strength of 0.1M) was shaken with the organic phase of equal volume for 20 hours except for U(IV), which was shaken only 30 minutes, at 25°C. Equilibrium between the phases was reached within this time. The phases were separated by centrifugation and aliquots were removed for the determination of the metal concentration in each phase and the pH of the aqueous phase.

The Sr and Hf concentrations were determined radiometrically using the isotopes Sr^{89} and Hf^{181} . The radioactivity (I'_{org} and I'_{aq} counts per minut (cpm)) of 10 ml of each phase was measured with a liquid-type GM-tube (Type M6, 20th Century Electronics, London) and a standard counting circuit. As the efficiency of the counting varies with the density of the solutions, and the type and energy of radiation, the measured distribution ratio ($q' = I'_{\text{org}}/I'_{\text{aq}}$) was multiplied by a correction factor (1/R, cf. [12]). The correction factors used for Hf were 1/0.89 for CHCl_3 and 1/1.05 for $\text{C}_4\text{H}_9\text{COCH}_3$. The results for Sr and Hf are given in Tables 4 and 5.

Table 4. The percentage extraction E of Sr(II) from 0.1 M NaClO_4 into CHCl_3 at 25°C. $[\text{Sr(II)}]_{\text{aq}}^0 = 10^{-3}\text{M}$. All concentrations in moles/l.

$[\text{HAA}]_{\text{org}}^0$	pH	$p\text{Aa}$	I'_{org} , cpm	I'_{aq} , cpm	$q' \approx q$	E
0.99	1.99	8.22	6 ± 5	3750 ± 20	< 0.003	< 0.3
0.99	3.56	6.65	-3 ± 5	3610 ± 20	< 0.001	< 0.1
0.99	5.98	4.23	3 ± 5	3690 ± 20	< 0.002	< 0.2
0.99	9.74*	0.83*	-4 ± 5	3530 ± 20	< 0.001	< 0.1

* Probably some decomposition of HAA (cf. [33]).

Table 5a. The percentage extraction E of Hf(IV) from 0.1M NaClO_4 into CHCl_3 at 25°C in the presence of acetylacetone (HAA). $[\text{Hf}]_{\text{aq}}^0 \leq 2.6 \times 10^{-5}\text{M}$. All concentrations in moles/l.

$[\text{HAA}]_{\text{org}}^0$	pH	$p\text{Aa}$	I'_{org} , cpm	I'_{aq} , cpm	q'	q	E
0.050	2.81	8.70	46 ± 5	1556 ± 40	0.030	0.034	3.3
"	3.13	8.38	101 ± 8	1514 ± 40	0.067	0.075	7.0
"	3.42	8.09	34 ± 4	416 ± 25	0.082	0.092	8.4
"	3.96	7.55	149 ± 10	570 ± 30	0.26	0.29	23 ± 3
"	4.00	7.51	37 ± 4	191 ± 20	0.19	0.21	17 ± 6
"	5.61	5.90	414 ± 20	78 ± 7	5.31	6.01	84 ± 10
"	7.19	4.32	1218 ± 30	75 ± 7	16.2	18.2	95 ± 10
"	7.38	4.13	1078 ± 30	65 ± 6	16.6	18.6	95 ± 10
"	7.85	3.66	391 ± 20	54 ± 5	7.24	8.14	89 ± 10
"	8.33	3.19	311 ± 15	72 ± 7	4.32	4.85	83 ± 10
"	8.79	2.74	839 ± 35	817 ± 35	1.026	1.15	54 ± 5
"	9.04	2.50	143 ± 10	367 ± 25	0.39	0.44	31 ± 3
0.005	3.10	9.41	5 ± 5	1586 ± 40	~ 0	—	~ 0
"	5.19	7.32	3 ± 5	1630 ± 40	~ 0	—	~ 0
"	5.86	6.45	-2 ± 5	1481 ± 40	~ 0	—	~ 0
"	7.91	4.60	4 ± 5	1528 ± 40	~ 0	—	~ 0

Table 5b. The percentage extraction E of Hf(IV) from 0.1M NaClO₄ into hexone at 25°C in the presence of acetylacetone (HAA). $[\text{Hf}]_{\text{aq}}^0 \leq 2.6 \times 10^{-5} M$. All concentrations in moles/l.

$[\text{HAA}]_{\text{org}}^0$	pH	$p\text{Aa}$	I'_{org} , cpm	I'_{aq} , cpm	q'	q	E
0.050	2.88	8.09	53 ± 6	2342 ± 40	0.023	0.022	2.2
"	2.98	7.99	31 ± 5	1821 ± 40	0.017	0.016	1.6
"	3.42	7.55	40 ± 5	1626 ± 40	0.025	0.024	2.4
"	4.48	6.49	62 ± 6	1307 ± 35	0.048	0.046	4.4
"	6.58	4.39	149 ± 5	395 ± 12	0.38	0.36	26
"	8.42	2.57	59 ± 6	674 ± 15	0.088	0.084	7.7
"	8.97	2.06	42 ± 5	2213 ± 40	0.019	0.018	1.8
"	9.46	1.76	38 ± 5	4780 ± 50	0.008	0.008	0.8
"	9.66	1.61	16 ± 5	4519 ± 50	0.004	0.004	0.4
0.005	3.22	8.75	-2 ± 5	1704 ± 40	~ 0	—	~ 0
"	3.88	8.09	-2 ± 5	287 ± 20	~ 0	—	~ 0
"	7.05	4.92	0 ± 5	151 ± 13	~ 0	—	~ 0
"	7.80	4.17	-6 ± 5	396 ± 25	~ 0	—	~ 0

The distribution of the fission products was followed by measuring the mixed β - and γ -activity with an internal gas-flow proportional counter (Model PC3, Nuclear Measurements Corp., Indianapolis). The activity from uranium, its disintegration products and Pu²³⁹ in the samples could be neglected compared to the activity of the fission products (cf. Table 1, ref. [20]). The results are given in Table 6.

Table 6. The percentage extraction E of β - and γ -activity from neutron irradiated uranium into C₆H₆ from 0.1M NaClO₄ at 25°C in the presence of acetylacetone (HAA). Irradiation time = cooling time = 1 year. $[\text{FP}]_{\text{aq}}^0 < 10^{-8} M$. All concentrations in moles/l.

$[\text{HAA}]_{\text{org}}^0$	pH	$p\text{Aa}$	I'_{org} , cpm	I'_{aq} , cpm	q	E
0.70	< 2.46	> 7.36	0 ± 5	≥ 5000	$\leq 10^{-3}$	< 0.1
"	3.42	6.40	0 ± 30	1158	≤ 0.03	≤ 3
"	3.77	6.05	608 ± 40	890 ± 50	0.67 ± 0.10	41 ± 5
"	3.83	5.99	589 ± 40	730 ± 50	0.81 ± 0.10	45 ± 4
"	5.59	4.23	1060 ± 60	469 ± 40	2.26 ± 0.3	69 ± 3
"	5.81	4.01	1085 ± 60	431 ± 40	2.52 ± 0.3	72 ± 3
"	6.14	3.68	983 ± 60	390 ± 40	2.51 ± 0.3	72 ± 3
"	6.27	3.55	1173 ± 60	513 ± 40	2.28 ± 0.3	70 ± 3
"	7.44	2.38	1136 ± 60	534 ± 40	2.12 ± 0.3	68 ± 3

The U(IV) concentration was determined by the same method as described in ref. [14]. The results are given in Table 7, where $[\text{U}_t]$, $[\text{U}_4]$ and $[\text{U}_6]$ refer to the concentrations of total uranium, U(IV) and U(VI), respectively. The distribution of U(IV), q_4 , has been calculated using the expression

$$q_4 = \frac{[\text{U}_4]_{\text{org}}}{[\text{U}_4]_{\text{aq}}} = \frac{[\text{U}_t]_{\text{org}} - q_6([\text{U}_t]_{\text{aq}} - [\text{U}_4]_{\text{aq}})}{[\text{U}_4]_{\text{aq}}}$$

Table 7. The percentage extraction E of U(IV) from 0.1M NaClO₄ into CHCl₃ at 25°C in the presence of acetylacetone (HAa); [HAa]_{org}⁰ = 0.50M. All other concentrations in millimoles/l.

pH	pAa	[U] _{aq}	[U] _{aq}	q_6	[U] _{org}	[U] _{org}	q_4	E_4	(E_4) _{corr}
1.97	8.54	0.961	0.839	≤ 0.01	0.071	0.071	0.084	8	8
2.16	8.35	0.796	0.673	< 0.01	0.233	0.232	0.345	26	26
2.38	8.13	0.631	0.511	0.017	0.386	0.384	0.75	43	43
2.48	8.03	0.538	0.392	0.025	0.527	0.524	1.34	57	57
2.52	7.99	0.520	0.401	0.029	0.513	0.510	1.25	55	61
2.68	7.83	0.382	0.262	0.055	0.678	0.671	2.56	72	80
2.87	7.64	0.253	0.144	0.11	0.756	0.744	5.16	84	90
3.04	7.47	0.232	0.129	0.22	0.812	0.790	6.12	86	94
3.04	7.47	0.279	0.140	0.22	0.777	0.748	5.35	84	94
3.26	7.25	0.155	0.066	0.44	0.922	0.833	13.4	93	98

In Table 7, (E_4)_{corr} gives the percentage of U(IV) extracted, when corrections for carbonate complexing have been made [14] (the pH was adjusted with NaHCO₃), assuming that no complexes are formed between U(IV) or U(VI) and benzene or chloroform [21]. A constant of $10^{4.0 \pm 0.2}$ is estimated [21] for the distribution of UAa₄ between CHCl₃ and H₂O.

The potassium concentration was determined gravimetrically after the evaporation of the organic phase. The results are given in Table 8.

Table 8. The percentage extraction E of K(I) from H₂O into CHCl₃ at 25°C in the presence of acetylacetone (HAa). [ClO₄⁻] = 0.1M. All concentrations in moles/l.

[HAa] _{org} ⁰	pH	pAa	[K] _{org}	[K] _{aq}	q	E
0.50	3.12	7.39	< 0.0003	0.10	< 0.003	< 0.3
"	4.65	5.86	< 0.0003	0.10	< 0.003	< 0.3
"	7.91	2.60	< 0.0003	0.10	< 0.003	< 0.3

As in previous papers of this series pH and pAa are used as abbreviations for $-\log[H^+]$ and $-\log[Aa^-]$. pAa is calculated from the equation

$$pAa = -\log[Aa^-] = pk_a - pH - \log m + \log(v_{aq} + k_d \cdot v_{org} + k_a \cdot v_{aq}[H^+]^{-1}),$$

where k_d is the distribution constant of HAa between the organic and aqueous solvents of equilibrium volumes v_{org} and v_{aq} respectively. The number of moles m of Aa in the system, is given by

$$m = [HAa]_{aq} \cdot v_{aq} + [Aa^-] \cdot v_{aq} + [HAa]_{org} \cdot v_{org},$$

if the amount of the metal complexes with HAa are negligible compared with m . [HAa]_{org}⁰ denotes the initial concentration of HAa in the organic phase. Thus, if no HAa has previously been added to the aqueous phase [HAa]_{org}⁰ = m/v_{org} .

The experimental results given in Tables 4–8 are summarized in Tables 1–3.

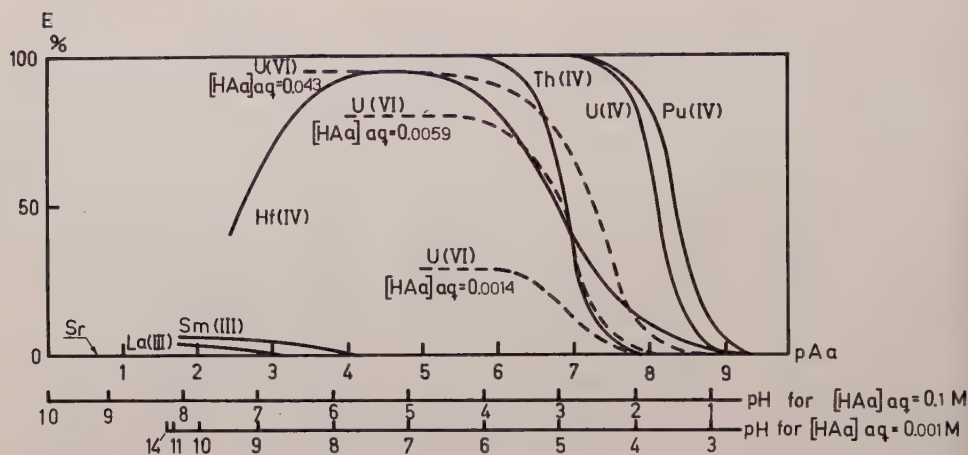


Fig. 1. The percentage E of some metals extracted into CHCl_3 from $0.1M$ NaClO_4 solutions of varying acetylacetone (HAA) concentrations; $pAa = -\log[Aa^-]$. The pH abscissae refer to a system of equal phase volumes and an initially (a) $2.45M$ HAA solution, (b) $0.0245M$ HAA solution in CHCl_3 ($[HAA]_{\text{org}}^0$); the equilibrium concentrations of HAA in the aqueous phases are (a) $0.1M$, (b) $0.001M$, if $[HAA]_{\text{aq}}^0 = 0$. The constants used for the relation between pH and pAa are $pK_a = 8.82$ and $k_d = 23.5$ [22].

Results and discussion of experiments

Separation of metals by extraction into chloroform

The percentage of a certain metal extracted into CHCl_3 from aqueous NaClO_4 solutions of varying pH and acetylacetone concentrations are given in Fig. 1. The experimental conditions used to obtain these extraction curves are summarized in Table I.

It can be estimated [16] that at $pAa < 2$ more than 50 % of the Sm(III) and La(III) will be hydrolysed, while hydrolysis can be neglected at $pAa > 3$. It would therefore be expected that the Sm and La curves could reach a value of E of at least twice that in Fig. 1, if the HAA concentration is considerably increased.

The extraction curves for tetravalent Th , U and Pu depend only on $[Aa^-]$ as long as $pH < 7$. The extraction of Ce reported by STENE [5] probably refers to Ce(IV) , which should be extracted to about the same extent as the tetravalent actinides, while Ce(III) would not be extracted much better than La(III) or Sm(III) .

Assuming a value of 10^{11} for the formation constant of HfOH^{3+} from Hf^{4+} and OH^- (cf. [23]), it can be calculated that Hf is extensively hydrolysed even at the lowest value of pH investigated, corresponding to $pAa \sim 9$ (cf. Fig. 1). The variation of E with $[HAA]$ at constant $[Aa^-]$ (cf. Table 5a) can therefore be explained by this fact. From studies of the distribution of a number of tetravalent elements between benzene and water in the presence of thenoyltrifluoroacetone ("TTA") [24] it is known that (probably) unhydrolysed Hf is extracted up to higher acidities (i.e. higher pAa values) than unhydrolysed Pu , U and Th . There is no reason to expect any change in this order,

when acetylacetone is used instead of thenoyltrifluoroacetone. It is also seen in Fig. 1 that the right-hand end of the Hf curve cuts through the curves for tetravalent Th, U and Pu. If the hydrolysis of Hf was negligible, as it may be when the aqueous phase is saturated with acetylacetone ($[HAA]_{aq} = 1.7M$), it seems reasonable to assume that the Hf curve would fall to the right of and be parallel to the Pu(IV) curve.

No extraction was obtained with K^+ or Sr^{2+} .

The variation of the U(VI) curve with $[HAA]$ at constant $[Aa^-]$ is at the lowest HAA concentration partially due to hydrolysis, while at the higher $[HAA]$ values it is due to the formation of an additive complex with uncharged HAA , UO_2Aa_2HAA [13].

These results on U(VI) seem to contradict the findings of STENE [5]. However, STENE's failure to extract U(VI) into CCl_4 or $CHCl_3$ may be due to the fact that he uses an acetate buffer of pH 4.5. It is known that U(VI) forms fairly strong complexes with acetate. The formation constant for the reaction ($Ac^- =$ acetate ion)



is around 2×10^6 [25, 26]. Thus at pH 4.5 practically all the U(VI) is bound as acetate complexes, which are not extracted into the organic solvent. However, at $pH > 7$ some U(VI) should have been extracted, since the U(VI) acetylacetonate complexes dominate over the acetate complexes under these experimental conditions.

If it is assumed that Abrahamczik's uranium is U(VI) and that the distribution ratio of uncharged uranyl acetylacetonate between CCl_4 and water is about the same as between $CHCl_3$ and water, it is surprising that ABRAHAMCZIK [6] reports the extraction of U from aqueous bicarbonate solutions of $pH \sim 7$ into CCl_4 , since the bicarbonate complexes of U(VI) are thought to be much stronger than the acetate complexes [27].

In order to clarify this somewhat confusing situation, the following experiments were carried out:

Aqueous solutions containing $NaHCO_3$ and U(VI) were shaken at $25^\circ C$ for 3 hours with equal volumes of CCl_4 or $CHCl_3$ containing acetylacetone. After the phases had been separated by centrifugation, the uranium in the aqueous phase was determined spectrophotometrically [28]. The results and experimental conditions are given in Table 9. It is seen that practically no U(VI) is extracted into CCl_4 or $CHCl_3$ at low HAA concentrations. At high concentrations of HAA , the acetylacetone complexes dominate over the carbonate complexes, which makes it possible to extract some of the U(VI) acetylacetonate.

Table 9. The distribution of U(VI) between organic solvents and $0.1M NaHCO_3$ in the presence of acetylacetone (HAA) at $25^\circ C$. All concentrations in moles/l.

Organic solvent:	CCl_4	CCl_4	$CHCl_3$
$[HAA]_{org}$	2.0	0.1	0.1
$[U]_{org}$	0.32×10^{-3}	$\leq 0.01 \times 10^{-3}$	$\leq 0.03 \times 10^{-3}$
$[U]_{aq}$	0.68×10^{-3}	$(1.01 \pm 0.02) \times 10^{-3}$	$(0.99 \pm 0.02) \times 10^{-3}$
percentage U extracted (E)	32	≤ 1	≤ 3
pH	7.6–7.8	8.03	8.06

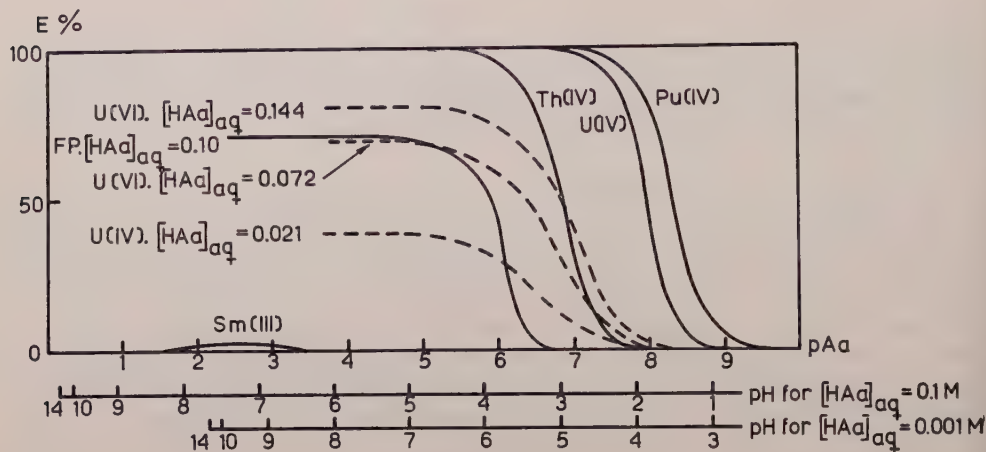


Fig. 2. The percentage E of some metals extracted into C_6H_6 from $0.1M$ $NaClO_4$ solutions of varying acetylacetone (HAA) concentrations; $pAa = -\log[Aa^-]$. The pH abscissae refer to a system of equal phase volumes and an initially (a) $0.70M$ HAA solution, (b) $0.007M$ HAA solution in C_6H_6 ($[HAA]_{org}^0$); the equilibrium concentrations of HAA in the aqueous phases are (a) $0.1M$, (b) $0.001M$, if $[HAA]_{aq}^0 = 0$. The constants used for the relation between pH and pAa are $pK_a = 8.82$ [22] and $k_d = 5.95$ [19].

Note.—The Th curve refers to an aqueous solution of $0.01M$ $NaClO_4$.

From these considerations and Fig. 1 it can be concluded that Hf, U(VI) (and probably all hexavalent actinides) and the tetravalent actinides can easily be separated from the alkali metals, the alkaline earth and rare earth elements by extraction with acetylacetone into $CHCl_3$ (experimental conditions may be e.g. $pAa = 6$, $[HAA]_{aq} = 0.05M$, and consequently $pH = 4$). If the conditions for the extraction can be carefully controlled, several of the elements in these groups may be separated from each other, e.g. Pu(IV) from Th or U(VI) (at $pAa = 7.5$, $[HAA]_{aq} = 0.006M$ and $pH = 3.5$, about 95% Pu(IV) and 5% Th or U(VI) are extracted into $CHCl_3$ in one step). Under certain conditions the following elements may be extracted to some extent: the elements in Group IIIa except boron, the elements in the first transition series, Be, Cu(II) and Ce(IV).

Separation of metals by extraction into benzene

The percentage of Sm(III), Th(IV), U(IV), Pu(IV), U(VI) and FP extracted from $0.1M$ $NaClO_4$ into C_6H_6 is given in Fig. 2, and the experimental conditions are summarized in Table 2. It is seen that the same general picture is obtained as for $CHCl_3$ in Fig. 1.

Sm is also partially hydrolysed under these conditions (about 50% at $pAa = 2$); with increasing $[HAA]$ the percentage extracted will probably increase somewhat.

No investigation of K^+ or Sr^{2+} has been made, but it can be assumed that the extraction of these elements into C_6H_6 can be neglected.

It is somewhat easier to separate U(VI) from a tetravalent actinide by extraction into benzene than into chloroform, since at the same values of pAa and $[HAA]_{aq}$ U(VI)

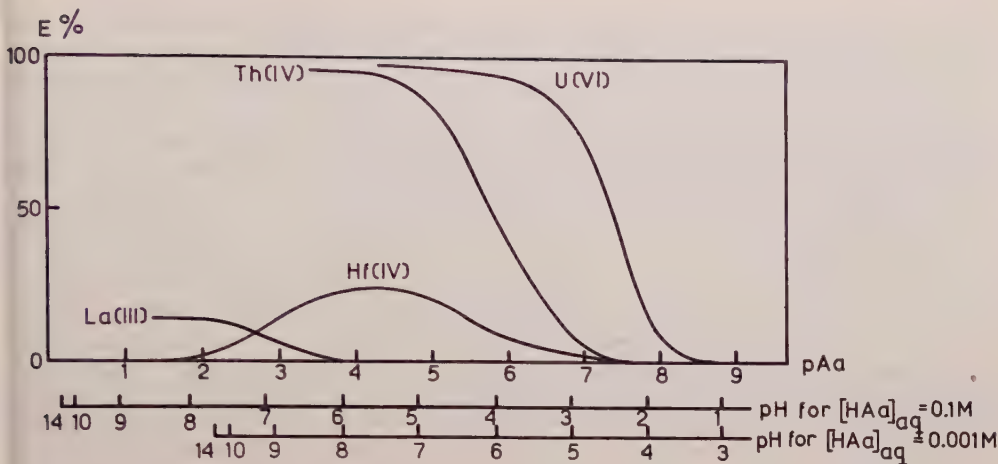


Fig. 3. The percentage E of some metals extracted into $C_4H_9COCH_3$ (hexone) from $0.1M$ $NaClO_4$ solutions of varying acetylacetonate (HAA) concentrations; $pAa = -\log[Aa^-]$. The pH abscissae refer to a system of equal phase volumes and an initially (a) $0.69M$ HAA solution, (b) $0.0069M$ HAA solution in hexone ($[HAA]_{org}^0$); the equilibrium concentrations of HAA in the aqueous phases are (a) $0.1M$, (b) $0.001M$, if $[HAA]_{aq}^0 = 0$. The constants used for the relation between pH and pAa are $k_a = 8.82$ and $k_d = 5.9$ [22].

is less easily extracted into benzene than it is into chloroform, while the tetravalent actinides extract equally well into both solvents.

It will be about equally easy to separate U(VI) and the tetravalent actinides together from the rare earth and alkaline earth elements by the extraction into C_6H_6 as by the extraction into $CHCl_3$. It is seen from Fig. 2 that at low acetylacetonate ion concentrations (low pH) no fission product activity is extracted into C_6H_6 ; in this region it will be possible to separate the tetravalent actinides from most of the U(VI) and the fission products.

Separation of metals by extraction into hexone

The percentage of La(III), Hf(IV) and U(VI) extracted into hexone from $0.1M$ $NaClO_4$ solutions as a function of pAa is shown in Fig. 3. The experimental conditions used to obtain these curves are given in Table 3.

It can be shown that the hydrolysis of La [16], U(VI) and Th [13] can be neglected under these conditions, while Hf is strongly hydrolysed. As pointed out in a previous section, the extraction curve for unhydrolysed Hf is considered to fall to the right of the Th curve. That this should be so is also indicated by the right-hand end of the Hf curve, which cuts the Th curve at the lowest pH (highest pAa) values.

If the curves for Hf in Fig. 1 and Fig. 3 are compared, it is seen that the general shape of the two curves is the same, and that the maximum percentage extraction (E_{max}) is obtained at the same pAa value (~ 4.5) in both cases. This indicates that similar processes (i.e. hydrolysis competing with complex formation with HAA) take place in the aqueous phase (cf. [17]). E_{max} has a higher value in $CHCl_3$ than in hexone, as also is the case for Th(IV).

There is a distinct difference between the relative positions of the Th and U(VI) curves in hexone and in benzene or chloroform. While Th(IV) is more easily extracted into benzene or chloroform than is U(VI), U(VI) is more easily extracted into hexone than is Th(IV). The reason for this seems to be that the organic solvent hexone forms a complex with the charged thorium acetylacetonate molecules, which hinders the formation of uncharged ThAa_4 (for details, see ref. [13] and [17]). No such complexes seem to be formed with U(VI).

If the results for U(VI), Th(IV) and La(III) are considered to be typical for all hexavalent and tetravalent actinides and rare earth elements, respectively, Fig. 3 indicates that it should be easy to separate the hexavalent and tetravalent actinides from the rare earth (and probably also from the alkaline earth) elements by extraction of the former into hexone. U(VI) and Th(IV) can also be separated from each other, though not completely in one step (e.g. at $\text{pAa } 7.2$, $[\text{HAA}]_{\text{aq}} = 0.02M$ and $\text{pH } 3.3$ about 60 % U(VI) and 3 % of Th(IV) are extracted in one step into the organic phase; see also next section).

General considerations

In the previous discussion only single extraction steps have been considered. Since the distribution coefficients of the metals at some values of $[\text{Aa}^-]$ and $[\text{HAA}]_{\text{aq}}$ differ by at least a factor of 2, it will in general be possible to separate all the metals investigated from each other by repeated extraction. This can be done in many ways, e.g. by counter current distribution in a Craig multistage extraction machine [e.g. 29].

The solubility of the metal acetylacetonates in organic solvents do not in general exceed $0.1M$; at such high concentrations the complexes often polymerize in the solutions [e.g. 30]. The separation of metals by extraction with organic solvents and acetylacetone therefore seems more suitable for analytical than for preparative purposes.

The hydrolysis of the metal ions, which in previous sections have been discussed more as a disturbing phenomenon masking the pure acetylacetone effect on the extraction curves, may be used to advantage to achieve a better separation of two otherwise similar elements. This is exemplified by Hf in Fig. 1; it would have been much more difficult to separate Hf from Pu(IV) if Hf had not been considerably hydrolysed.

In a similar manner other metals, which have extraction curves very close to each other, can be well separated by extraction with acetylacetone, if one of these metals forms a non-extractable complex with an anion in the aqueous phase, as e.g. F^- , HSO_4^- , HCO_3^- or CH_3COO^- .

For example, sulfate is known to form stronger complexes with Th(IV) [31] than with U(VI) [32]. Thus if the extraction with acetylacetone into hexone is carried out in the presence of sulfate ions, the separation of Th(IV) from U(VI) will be better than is the case in the absence of sulfate ions (see Fig. 3). On the other hand, the sulfate concentration must be carefully controlled, since too high sulfate concentrations will make it impossible to extract U(VI).

The disturbing action of the complex forming anions HCO_3^- and CH_3COO^- on the extraction of U(VI) has been discussed previously in connection with Fig. 1.

The previous discussion on the separation of metals by solvent extraction in the presence of acetylacetone has been based on single extraction curves for individual metals, and it has tacitly been assumed that in the presence of several metals the individual extraction curves will be the same, so that the metals do not interfere with each other. This should be true as long as the activity coefficients in the solutions are kept constant and the free ligand concentration in the solvent system is much larger than the concentration of all metals which form strong complexes with the ligand.

Summary

The possibility of separating metals by extracting some of them as acetylacetonates from aqueous NaClO_4 solutions into the organic solvents chloroform, benzene or methyl isobutyl ketone (hexone) is discussed in the light of recent information about the complex formation between the metals and acetylacetone. The following metals are discussed in greater detail: La(III), Sm(III), Hf(IV), Th(IV), U(IV), Pu(IV) and U(VI). The effect of anions such as OH^- , HSO_4^- , HCO_3^- and CH_3COO^- , which form non-extractable complexes with the metals, is also discussed.

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Studies on the extraction of metal complexes. XXV

The complex formation of Pu(IV) with acetylacetone

By JAN RYDBERG

With 3 figures in the text

The stability and distribution constants of the acetylacetone (HAa) complexes of Th(IV) and U(IV) have been reported previously in this series of investigations [1, 2]. Since the variation of these constants throughout the tetravalent actinide elements is of interest, the investigations have now been extended to Pu(IV).

The preparation of solid PuAa₄ has been reported by DIXON and SMITH [3], who investigated its vapor pressure at different temperatures. HARVEY, HEAL, MADDOCK, and ROWLEY [4] studied the distribution of tracer amounts of Pu(IV) between benzene and water in the presence of acetylacetone, and found that the distribution ratio increased from 0.5 at pH ~ 1 to an almost constant value of 80 at pH 5–10. No other investigations of plutonium acetylacetonate seem to have been reported.

1. Outline of the investigation

From studies of the distribution of a metal between an organic and an aqueous solvent as a function of metal concentration, pH, ligand concentration, etc., it is possible to identify the types of complexes formed in the two solvents. In many cases is also possible to calculate formation and distribution constants for the various complexes [5, 6]. This method of investigation, which is very suitable for low metal concentrations, has been used here as in previous investigations.

It is very difficult to prepare pure Pu(IV) in HClO₄ and to keep the plutonium in this valency state for more than a short time [7, 8], in the absence of stabilizing agents (complexing or redox). The use of such agents would necessitate an investigation of their complex formation with Pu(IV), and few such investigations have been reported [9, 10]. It was therefore considered that the distribution of Pu(IV) could be most conveniently studied in the absence of stabilizing agents, provided that it were possible to analyse all solutions for Pu(IV).

A number of different processes can be used to separate Pu(IV) from Pu(III), Pu(V) and Pu(VI) [cf. 11, 12]. The process selected utilised the extraction with 0.3*M* thenoyltrifluoroacetone ("TTA") in benzene from 1*M* HNO₃ solutions. Under these conditions only Pu(IV) is extracted into the benzene phase [12, 13]. The benzene phase could then be evaporated and the amount of Pu determined by measuring the α -activity of the sample.

2. Experimental

a. General procedure

The aqueous phase was prepared by adding in turn solutions of HClO_4 , NaOH , H_2O , acetylacetone and 0.01–0.05 ml of $\sim 10^{-3}M$ plutonium perchlorate to make up a 3 ml phase of ionic strength $0.1M$ and of a suitable pH. This phase was then mixed in a 15 ml centrifuge tube with 3 ml benzene by vigorous stirring for 10 minutes at 25°C . (Identical results obtained after 5, 10 and 15 minutes stirring indicated that equilibrium between HAA and the Pu species of different valency states is reached within this time, although equilibrium may not be reached between the different valency states of Pu. However, the attainment of the latter equilibrium is of no importance in the present investigation.) After the phases had been separated by centrifuging, samples of each phase were withdrawn for analysis of Pu(IV) and for pH determination.

As in previous investigations pH is used as the symbol for $-\log [\text{H}^+]$. The pH was measured with a valve potentiometer (pH-Meter 3i, Radiometer, Copenhagen), which had been calibrated against solutions of known hydrogen ion concentration.

b. Determination of Pu(IV)

The analytical procedure is outlined in Fig. 1. Each phase is numbered and the same number is used as a superscript to denote the radioactivity of that phase, I counts per minute per ml (cpm/ml). The valency states of plutonium, which may be present in each phase, are also indicated. The radioactivity of plutonium in a certain valency state is indicated by a subscript. Thus for the distribution ratio, q_{IV} , of Pu(IV) in the original acetylacetone system, $q_{\text{IV}} = I_{\text{IV}}^{(1)}/I_{\text{IV}}^{(11)}$. In the following discussion it will be assumed in the first instance that all squares in Figure 1 containing plutonium refer to equal volumes; thus $c_{\text{IV}}^{(1)} = c_{\text{IV}}^{(3)} = c_{\text{IV}}^{(4)} + c_{\text{IV}}^{(5)}$, $c_{\text{IV}}^{(5)} = c_{\text{IV}}^{(6)} + c_{\text{IV}}^{(7)}$, etc., where c = concentration and assuming a 100 % mass balance.

All the extractions with $0.3M$ TTA in benzene are carried out on $1M$ HNO_3 solutions. It was found [13] that under these circumstances $> 95\%$ of Pu(IV) , $< 1\%$ of Pu(VI) , practically no Pu(III) and very probably no Pu(V) is extracted into the benzene phase ($q_{\text{IV}}^{\text{TTA}} > 20$, $q_{\text{VI}}^{\text{TTA}} < 0.01$, $q_{\text{III}}^{\text{TTA}}$ and $q_{\text{V}}^{\text{TTA}} < 10^{-4}$, where q_i^{TTA} represents the distribution ratio of Pu_i between $0.3M$ TTA in benzene and $1M$ HNO_3).

It can be shown that

$$\frac{I^{(4)}}{I^{(14)}} = \frac{q_{\text{IV}}^{\text{TTA}} (1 + q_{\text{VI}}^{\text{TTA}}) c_{\text{IV}}^{(1)} + q_{\text{VI}}^{\text{TTA}} (1 + q_{\text{IV}}^{\text{TTA}}) c_{\text{VI}}^{(1)}}{q_{\text{IV}}^{\text{TTA}} (1 + q_{\text{VI}}^{\text{TTA}}) c_{\text{IV}}^{(11)} + q_{\text{VI}}^{\text{TTA}} (1 + q_{\text{IV}}^{\text{TTA}}) c_{\text{VI}}^{(11)}} \quad (1)$$

With the values given above for $q_{\text{IV}}^{\text{TTA}}$ and $q_{\text{VI}}^{\text{TTA}}$ (Pu(III) and Pu(V) can be neglected here) one finds that, for $c_{\text{IV}} \geq c_{\text{VI}}$, this expression can be approximated to

$$\frac{I^{(4)}}{I^{(14)}} = \frac{c_{\text{IV}}^{(1)}}{c_{\text{IV}}^{(11)}} = q_{\text{IV}} \quad (2)$$

Under these circumstances the distribution ratio of Pu(IV) between benzene (phase 1) and water (phase 11) in the presence of acetylacetone is easily obtained. However,

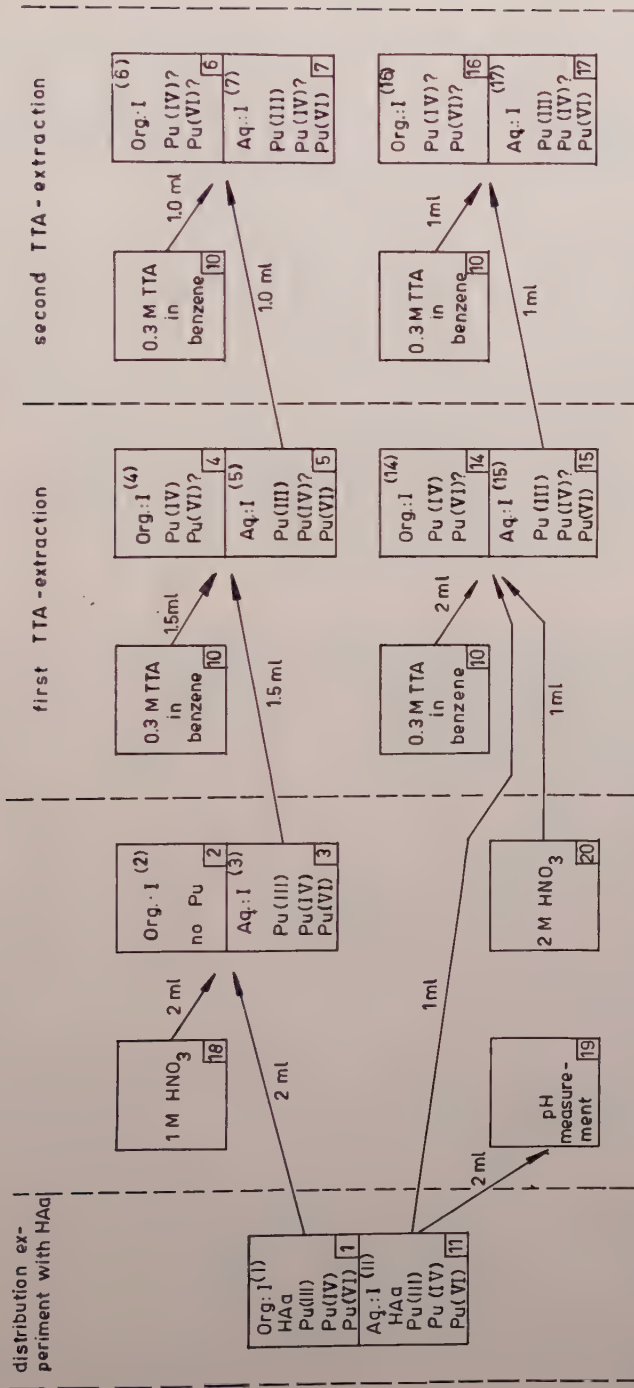


Fig. 1. Analytical flow-sheet for determining the distribution ratio q_{IV} of Pu(IV) between organic (phase I) and aqueous (phase II) solutions.

if the experimental conditions are such that $c_{VI} > c_{IV}$, which may happen in the aqueous phase, the validity of eq. (2) must be proved by carrying out a second extraction with TTA.

If an aqueous phase (e.g. phase 3) is repeatedly extracted (say n times) with equal volumes of new organic phase (e.g. phase 10), it can be shown [cf. 14] that the percentage $(E)_n$ of the solute obtained in the organic solvent at the n th extraction is

$$(E)_n = q(1+q)^{-n} 100. \quad (3)$$

For the first TTA extraction of Pu(IV) one obtains

$$c_{IV}^{(4)} = c_{IV}^{(3)} (E_{IV})_1. \quad (4 a)$$

and for the second TTA extraction of Pu(IV) one obtains

$$c_{IV}^{(6)} = c_{IV}^{(3)} (E_{IV})_2. \quad (4 b)$$

The ratio between the radioactivities from plutonium in phases 6 and 4 is given by

$$\frac{I^{(6)}}{I^{(4)}} = \frac{c_{IV}^{(4)} (E_{IV})_2 (E_{IV})_1^{-1} + c_{VI}^{(4)} (E_{VI})_2 (E_{VI})_1^{-1}}{c_{IV}^{(4)} + c_{VI}^{(4)}}. \quad (5)$$

Assuming that the separation of Pu(IV) and Pu(VI) in the extraction with TTA is less good than previously stated, say $q_{IV}^{TTA} \geq 10$ and $q_{VI}^{TTA} \leq 0.1^*$, it follows from equations (3) and (5) that, if phase 4 contains 10 % of Pu(VI), the ratio $I^{(6)}/I^{(4)}$ will be 0.16 (for 20 % of Pu(VI) it will be 0.23). Provided that the ratio is < 0.16 , the error in setting $I^{(4)} = I_{IV}^{(4)}$ is < 10 %. On the other hand, with $q_{IV}^{TTA} = 10$ only 90 % of the Pu(IV) is extracted, but this extra error of 10 % works in the opposite direction. The total error in setting $I^{(4)} = \text{constant} \cdot c_{IV}^{(1)}$ will therefore be less than ± 10 %.

A similar argument for phases 14 and 16 leads to an analogous result.

It can therefore be concluded that as long as $I^{(6)}/I^{(4)}$ or $I^{(16)}/I^{(14)}$ is < 0.16 , the maximum error in using eq. (2) for calculating the distribution ratio of Pu(IV) between benzene and 0.1M NaClO₄ in the presence of acetylacetone will be less than ± 20 %, which corresponds to an error in $\log q_{IV}$ of ± 0.08 log units (for a ratio of 0.23, the maximum error in $\log q_{IV}$ will be ± 0.15). The ratios $I^{(6)}/I^{(4)}$ and $I^{(16)}/I^{(14)}$ are given in Tables 1-3.

In order to test if the equilibrium between the different valency states of plutonium changed during the analytical procedure, part of phase 3 was extracted with fresh TTA in benzene (phase 10) at certain time intervals. It was found that the activity extracted ($I^{(4)}$) at 5, 10 and 15 minutes (counted from the moment phase 3 had been prepared) was constant, while after 30 minutes it had considerably increased. Similar tests on phases 14 and 15 in contact with each other gave analogous results. In the latter experiments, the amount extracted ($I^{(14)}$) was higher than in the former experiment (phase $I^{(4)}$) after 30 minutes. This behavior may be explained by a shift in the equilibrium between the different valency states of plutonium towards Pu(IV), since Pu(IV) is favoured by high acidities [10] and also by the presence of strong complexing agents such as TTA.

* For the extraction of Pu with TTA a range can always be selected where $q_{IV}^{TTA} \cdot q_{VI}^{TTA} = 1$.

As a consequence of these observations, the analyses were carried out as quickly as possible. The time required for the isolation of Pu(IV) in one phase (i.e. all experiments from the isolation of phase 1 to phase 6, or phase 11 to phase 16) was less than 10 minutes. All the results given in Tables 1-3 have been obtained in this manner. If the analytical procedure is slowed down to half an hour or more, the increase in c_{IV} will lead to too low q_{IV} values. This effect is most prominent at high values of q_{IV} .

The samples for the measurements of α -activity were obtained by withdrawing 0.1 ml of each phase with a pipet, and then slowly evaporating the solution on a platinum dish placed on an aluminium ring on top of a hot plate at 130°C. The pipets were washed with 1M HNO₃, which dissolves the plutonium adsorbed on the glass walls (cf. [15]); these washings were added to the platinum dish. No visible amounts could be detected on the Pt-dishes after the evaporation. It has been pointed out [16] that the Np(IV) TTA compound may volatilize during evaporation, and in order to avoid this the addition of trichloroacetic acid has been recommended. However, the same results were obtained when the Pu(IV) TTA was evaporated with and without the addition of trichloroacetic acid. The samples were measured either in the internal chamber of a proportional counter (Model PC-3, Nuclear Measurements Corporation, Indianapolis) or with a scintillation counter connected to an automatic sample changer [17].

c. Chemicals

Plutonium was isolated from neutron-irradiated uranium by a combination of sorption, precipitation and extraction processes, which have been described in detail elsewhere [18]. During the separation process the amounts of uranium and fission products were decreased by a factor of 10^6 relative to plutonium, so that no interference from these elements would occur with the α -measurements on plutonium. Plutonium perchlorate was prepared by evaporating a $10^{-3}M$ solution of plutonium in 10M HNO₃ almost to dryness and then diluting with 5M HClO₄ to approximately the original volume. Such solutions were prepared repeatedly in order to have fresh perchlorate solutions which contained mainly Pu(IV).

Acetylacetone (Riedel de Haen, für Chromatographie) was purified as described before [19]. Several attempts were made to purify the thenoyltrifluoroacetone, TTA, (The Dow Chemical Company, Pittsburg, Cal.) by recrystallization, vacuum sublimation, etc., but the products thus obtained all gave the same melting points as the original Dow product. The TTA was therefore used without any additional purification, and dissolved in benzene to make an 0.3M solution, which was then stored in darkness over a small volume of dilute nitric acid.

Benzene and chloroform of p.a. quality were washed with water several times before use. Methyl isobutyl ketone (hexone) of commercial grade was washed with acid, alkali and water to remove water soluble impurities. The water used was distilled once.

All other chemicals were of p.a. quality.

3. Results

a. Calculation of the distribution curve

The experimental results are given in Tables 1-3. It can be concluded from the measurements of the α -activity of the different organic phases and the discussion in section 2b that eq. (2) can be used for calculating q_{IV} . In order to simplify the above

Table 1. Calculation of the distribution ratio q_{IV} of Pu(IV) between benzene and 0.1M NaClO₄ at 25°C at different pH and acetylacetone (HAa) concentrations

[Pu] _{init} 10 ⁻⁶ M	[HAa] _{init} M	pH	-log [Aa ⁻]	$I^{(4)}$ cpm/ml	$I^{(6)}$ cpm/ml	$I^{(6)}/I^{(4)}$	$I^{(14)}$ cpm/ml	$I^{(16)}$ cpm/ml	$\frac{I^{(16)}}{I^{(14)}}$	log q_{IV}	Sym bol
~6	0.050	1.00	9.96	38.4	6.6	0.17	74 021	6 142	0.08	-3.59	●
~6	0.050	1.13	9.83	32	5.4	0.17	26 734	2 172	0.08	-3.22	●
~3	0.100	1.48	9.18	381	25	0.07	18 368	2 322	0.13	-1.98	●
~3	0.100	1.54	9.12	459	57	0.12	15 614	1 097	0.07	-1.83	●
~3	1.00	0.99	8.67	11 628	336	0.03	24 499	920	0.04	-0.62	○
~3	0.100	2.07	8.59	5 516	116	0.02	5 794	759	0.13	-0.32	●
~6	1.00	1.23	8.43	26 252	636	0.02	13 952	969	0.07	-0.03	○
~6	1.00	1.24	8.42	17 664	3 204	0.18	13 889	555	0.04	-0.20	○
~3	1.00	1.50	8.16	41 445	8 475	0.20	8 704	184	0.02	0.38	○
~3	0.100	2.77	7.89	57 652	1 400	0.02	6 766	1 148	0.17	0.63	●
~3	0.100	3.23	7.43	25 261	388	0.02	1 695	226	0.13	0.87	●
~6	1.00	2.40	7.26	87 422	1 039	0.01	1 930	165	0.09	1.35	●
~3	1.00	2.55	7.11	61 980	942	0.02	1 786	143	0.08	1.24	○
~6	1.00	3.08	6.58	83 840	1 370	0.02	940	150	0.16	1.65	●
~6	1.00	3.14	6.52	88 520	921	0.01	760	40	0.05	1.77	●
~6	1.00	3.15	6.51	98 670	550	0.01	1 570	30	0.02	1.50	●
~6	1.00	4.26	5.40	92 083	1 100	0.01	341	30	0.09	2.13	●
~6	1.00	5.14	4.52	87 580	530	0.01	250	31	0.12	2.24	●
~6	1.00	5.49	4.17	85 120	1 764	0.02	171	14	0.08	2.40	●
~3	0.100	7.06	3.60	81 790	11 590	0.14	106	6	0.03	2.59	●
~6	0.100	7.35	3.31	84 860	830	0.01	145	20	0.14	2.48	●
~3	1.00	6.54	3.12	69 060	536	0.01	64	10	0.16	2.73	●
~6	1.00	6.74	2.92	94 330	410	0.00	181	20	0.11	2.42	○
~6	1.00	6.85	2.81	73 018	419	0.01	114	19	0.16	2.51	●

Table 2. Calculation of the distribution ratio q_{IV} of Pu(IV) between chloroform and 0.1M NaClO₄ at 25°C at different pH and acetylacetone (HAa) concentrations

[Pu] _{init} 10 ⁻⁶ M	[HAa] _{init} M	pH	-log [Aa ⁻]	$I^{(4)}$ cpm/ml	$I^{(6)}$ cpm/ml	$I^{(6)}/I^{(4)}$	$I^{(14)}$ cpm/ml	$I^{(16)}$ cpm/ml	$\frac{I^{(16)}}{I^{(14)}}$	log q_{IV}	Sym bol
~3	1.00	1.29	8.92	9 100	130	0.01	36 830	1 920	0.05	-0.91	□
~3	1.00	2.62	7.59	55 620	490	0.01	2 830	320	0.11	0.99	□
~3	1.00	3.03	7.18	42 480	990	0.02	2 000	350	0.17	1.03	□
~3	1.00	6.54	3.67	69 826	7 100	0.10	87	13	0.15	2.60	□
~3	1.00	7.33	2.88	68 830	11 210	0.16	100	20	0.20	2.54	□

discussion, it was assumed that all liquid phases containing plutonium in Fig. 1 were of the same volume. However, phase 11 was actually diluted with an equal volume of 2M HNO₃ (phase 20). In order to make the specific activity measured in phases 4 and 14 comparable, $I^{(14)}$ must then be multiplied by a factor of 2; thus

$$q_{IV} = \frac{c_{IV}^{(I)}}{c_{IV}^{(II)}} = \frac{I^{(4)}}{2 I^{(14)}} \quad (6)$$

Eq. (6), and not eq. (2), has therefore been used to calculate q_{IV} in Tables 1-3.

Table 3. Calculations of the distribution ratio q_{IV} of Pu(IV) between methyl isobutyl ketone and 0.1M NaClO₄ at 25°C at different pH and acetylacetone (HAa) concentrations

$[Pu]_{init}$ $10^{-6}M$	$[HAa]_{init}$ M	pH	$-\log [Aa^-]$	$I^{(4)}$ cpm/ml	$I^{(6)}$ cpm/ml	$I^{(6)}/I^{(4)}$	$I^{(14)}$ cpm/ml	$I^{(16)}$ cpm/ml	$I^{(16)}/I^{(14)}$	$\log q_{IV}$	Sym-bol
~3	1.00	1.71	7.95	14 400	1 250	0.09	7 840	700	0.09	-0.04	△
~3	1.00	1.98	7.68	34 640	1 410	0.04	11 230	1 250	0.11	0.19	△
~3	1.00	2.19	7.47	41 960	1 930	0.05	6 430	800	0.12	0.51	△
~3	1.00	2.27	7.39	40 000	3 390	0.08	5 910	1 030	0.17	0.53	△
~3	1.00	3.52	6.14	61 160	1 330	0.02	2 100	140	0.07	1.16	△
~3	1.00	4.38	5.28	61 920	1 320	0.02	1 600	30	0.02	1.29	△
~3	1.00	7.50	2.16	33 450	1 660	0.05	910	150	0.16	1.27	△

The acetylacetonate ion concentration, $[Aa^-]$, has been calculated according to the equation

$$-\log[Aa^-] = pAa = pk_a - pH - \log[Aa]_{init} + \log(1 + k_d + k_a[H^+]^{-1}), \quad (7)$$

where k_a is the acid dissociation constant of HAa in 0.1M NaClO₄ ($k_a = 1.52 \times 10^{-9}$, $pk_a = 8.82$ [20]), k_d the distribution constant of HAa between the organic solvent and 0.1M NaClO₄ ($k_d = 5.8$ for benzene [19],¹ 5.9 for hexone [20] and 23.5 for chloroform [20]), and $[Aa]_{init}$ is the initial concentration of HAa and Aa⁻ in the aqueous phase, before any partition of HAa between the organic and aqueous phases has taken place. Owing to the low plutonium concentration ($[Pu]_{aq}$ and $[Pu]_{org} \leq 6 \times 10^{-6}M$), the amount of HAa bound to plutonium can be neglected.

b. Calculation of formation and distribution constants

It is seen from Fig. 2 that the distribution of Pu(IV) between benzene and 0.1M NaClO₄ does not show any systematic variation with the concentration of plutonium and acetylacetone at constant values of $[Aa^-]$. One can therefore conclude [5] that polynuclear complexes and complexes containing an excess of OH⁻ or undissociated HAa can be neglected; the complexes which have to be considered are therefore of the type $PuAa_n^{4-n}$. Similar complexes are formed by Th(IV) and U(IV) in the presence of acetylacetone [1, 2].

The distribution constants, λ_4 of the uncharged species $PuAa_4$ have been calculated from the horizontal part of the curves in Fig. 2. The few points obtained using chloroform seem to fall slightly above the points obtained using benzene. The values of the distribution constants are given in Table 5 together with the estimated errors.

Three different methods, referred to as the "two-parameter method" [21], the "ligand number method" [22] and the "limiting value method" [23], have been used in order to obtain the formation constants for the $PuAa_n^{4-n}$ complexes from the data for the benzene system given in Fig. 2.

¹ In other papers of this series, a value of 5.95 has been used, which actually refers to zero ionic strength. However, the difference of k_d can in fact be neglected, since it only corresponds to 0.01 pAa unit in eq. (7).

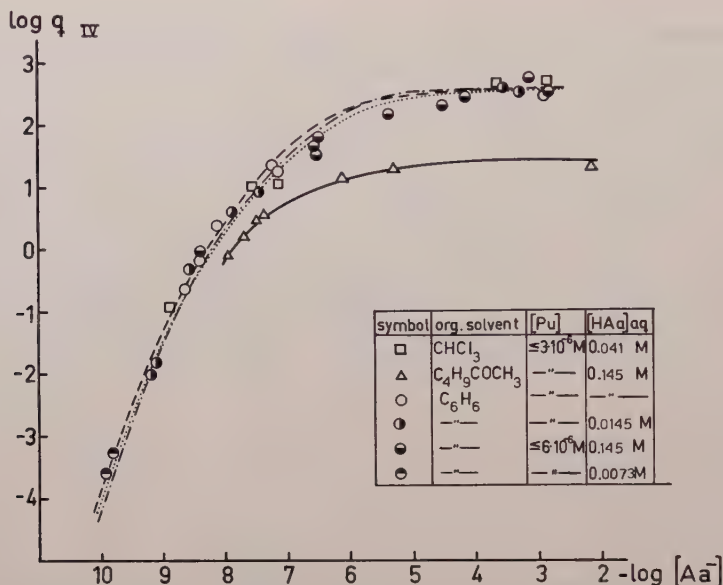


Fig. 2. The distribution ratio q_{IV} of Pu(IV) between benzene, chloroform or hexone and 0.1M NaClO₄ as a function of the acetylacetonate ion (Aa⁻) concentration. The broken curves are calculated with the constants obtained by the two-parameter method (---), the ligand number method (.....) and the limiting value method (-.-.-).

The two-parameter method is based on the approximation that the ratio between two successive step formation constants, k_n/k_{n+1} , is constant. Although this assumption is never exactly fulfilled in practice, the method is useful to obtain approximate values of the constants when the data are very scattered. With the values $a = 8.5$ and $b = 0.8$ for the two parameters, and $\log \lambda_4 = 2.44$, the overall complexity constant κ_n may be calculated from the equation [21]

$$\log \kappa_n = an + bn(4 - n). \quad (8)$$

κ_n is defined by

$$\kappa_n = \prod k_n, \quad (9)$$

where k_n is the stepwise formation constant. The values of κ_n and k_n are given in Table 4. The distribution curve calculated from these constants is indicated by the broken line (---) in Fig. 2. Other curves may be obtained using slightly different values of the two parameters a and b , and of λ_4 , which, however, fit the experimental data less well.

Since the two-parameter method did not give constants which reproduced the experimental data very well, the ligand number method was also used. The average number of ligands bound per central atom, $\bar{n}$, as a function of the ligand concentration was calculated from [1]

$$\bar{n} = 4 - d(\log q)/d(\log [Aa^-]) \quad (10)$$

Table 4. The overall formation constants κ_n and step equilibrium constant k_n calculated by different methods

Method	$\log \lambda_4$	$\log \kappa_1$	$\log \kappa_2$	$\log \kappa_3$	$\log \kappa_4$	$\log k_1$	$\log k_2$	$\log k_3$	$\log k_4$
Two-parameter method [21]	2.44	10.9	20.2	27.9	34.0	10.9	9.3	7.7	6.1
Ligand number method [22]	2.50	10.1	19.2	27.7	33.6	10.1	9.1	8.5	5.9
Limiting value method [23]	2.56	10.8+d*	19.4+d*	28.0+d*	34.0+d*	10.8+d*	9.6	8.6	6.0

* d probably ± 1 (see text)

Table 5. Equilibrium constants for the formation of PuAa_4^{4-n} in 0.1M NaClO_4 and distribution of PuAa_4 between C_6H_6 , CHCl_3 and $\text{C}_4\text{H}_9\text{COCH}_3$ and 0.1M NaClO_4 at 25°C

Reaction	Equilibrium constant
$\text{Pu}^{4+} + \text{Aa}^- \rightleftharpoons \text{PuAa}^{3+}$	$\log k_1 = 10.5 \pm 1$
$\text{PuAa}^{3+} + \text{Aa}^- \rightleftharpoons \text{PuAa}_2^{2+}$	$\log k_2 = 9.2 \pm 0.5$
$\text{PuAa}_2^{2+} + \text{Aa}^- \rightleftharpoons \text{PuAa}_3^+$	$\log k_3 = 8.4 \pm 0.5$
$\text{PuAa}_3^+ + \text{Aa}^- \rightleftharpoons \text{PuAa}_4$	$\log k_4 = 6.0 \pm 0.4$
$\text{PuAa}_4 (\text{Aq.}) \rightleftharpoons \text{PuAa}_4 (\text{C}_6\text{H}_6)$	$\log \lambda_4 = 2.5 \pm 0.2$
$\text{PuAa}_4 (\text{Aq.}) \rightleftharpoons \text{PuAa}_4 (\text{CHCl}_3)$	$\log \lambda_4 = 2.6 \pm 0.3$
$\text{PuAa}_4 (\text{Aq.}) \rightleftharpoons \text{PuAa}_4 (\text{C}_4\text{H}_9\text{COCH}_3)$	$\log \lambda_4 = 1.4 \pm 0.1$

by graphical differentiation of the best distribution curve through the points in Fig. 2 (this curve is not shown there). The resulting formation curve is given in Fig. 3. By taking four corresponding values of $\bar{n}$ and $[\text{Aa}^-]$ and substituting them into the equation [22]

$$\bar{n} + (\bar{n} - 1)\kappa_1[\text{Aa}^-] + (\bar{n} - 2)\kappa_2[\text{Aa}^-]^2 + (\bar{n} - 3)\kappa_3[\text{Aa}^-]^3 + (\bar{n} - 4)\kappa_4[\text{Aa}^-]^4 = 0 \quad (11)$$

four simultaneous equations were obtained and solved for κ_n . The values thus obtained are given in Table 4 together with the value of λ_4 assumed *a priori*. The distribution curve calculated with these values is indicated by the dotted curve (....) in Fig. 2.

An attempt was also made to calculate the formation constants by the limiting value method. With this method (cf. [1]) a set of constants

$$\varphi_{4-n} = \kappa_n \kappa_4^{-1} \quad (12)$$

may be obtained. Approximate values for φ_1 , φ_2 and φ_3 were fairly easily calculated. Since only few points were available at low values of q_{IV} , only a very approximate value of $\varphi_4 = 10^{-34+d}$ (i.e. $= \kappa_4^{-1}$, since $\kappa_0 = 1$) could be obtained, where d is an error of the order of ± 1 . Since the values of κ_n obtained by the limiting value method all depend on κ_4 (for $n \leq 4$), the uncertainty in d will affect the values of all the constants κ_n . However, it can be seen from eq. (9) and (12) that only k_1 of all the

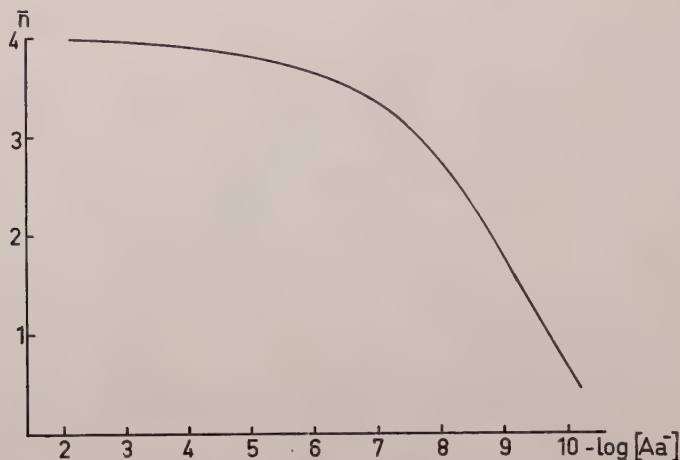


Fig. 3. The average number $\bar{n}$ of ligands bound to each Pu(IV) ion as a function of the ligand (acetylacetonate ion, Aa^-) concentration.

constants k_n will depend on φ_4 . The result of the calculations is given in Table 4 together with the value used for λ_4 . Assuming $d = 0$ these values have been used to calculate the distribution curve indicated by dots and dashes (- · - · -) in Fig. 2. The tail of the curve at low values of q_{IV} will depend on the values of d , i.e. a change of ± 1 in d will change $\log q_{IV}$ by approximately ± 0.35 at $\log [Aa^-] = -10$.

4. Discussion

It can be seen from Fig. 2 that none of the distribution curves obtained with the values of κ_n calculated by the different methods fit the experimental points exactly. This may be due to the experimental scatter of the points or to systematic errors in the analyses or errors in the assumptions on which the calculations are based. Of the three curves, the dotted one (obtained by the ligand number method) seems to fit the experimental data slightly better than the two other curves. Weighting the results of the ligand number method, the most probable values for the formation constants are given in Table 3, together with the distribution constants of the uncharged complex.

The formation constants in Table 5, with which very large errors are associated, should only be regarded as giving constants of the right order of magnitude. It would be most valuable if these formation constants could be determined by an independent method using macro amounts of plutonium.

If the formation constants for Th(IV), U(IV) and Pu(IV) with acetylacetone are compared (cf. ref. [1, 2]), it will be found that the constants increase in the order of decreasing ionic radius. However, the step formation constant k_4 for Pu(IV) deviates from this rule in that it is approximately equal to k_4 for U(IV). However, it is not clear if this effect is real or apparent due to the large uncertainty in the constant for Pu(IV).

The distribution constants λ_4 for the uncharged complex $PuAa_4$ are approximately the same for the benzene and the chloroform systems and considerably lower for the hexone system, as also was the case for $ThAa_4$ and UAa_4 . The distribution ratio $\log$

$\lambda_4 = 2.5$ obtained for the benzene–water system is higher than the value 1.9 given by HARVEY *et. al.* [4]. Since $q_{IV}^{TTA} > q_{VI}^{TTA}$ and $q_{IV}^{HAa} > q_{VI}^{HAa}$, a number of factors, such as a shift of the equilibrium towards Pu(IV) during the analyses or the presence of substances stabilizing Pu(IV), or hydrolyses of plutonium, all will tend to decrease q_{IV} rather than increase it, and consequently to give too low values of λ_4 . It is therefore believed that the higher value of λ_4 , i.e. $\log \lambda_4 = 2.5$, more correctly indicates the distribution ratio of $PuAa_4$ between benzene and 0.1M $NaClO_4$. Due to lack of information about the conditions for the extractions carried out by HARVEY *et. al.*, the reason for the discrepancy cannot be analyzed in detail.

Summary

The formation constants for the Pu(IV) acetylacetonate (Aa^-) complexes in 0.1M $NaClO_4$ at 25°C have been calculated from measurements of the distribution ratio of Pu(IV) between benzene and aqueous solutions containing acetylacetone. The distribution constants of uncharged $PuAa_4$ between benzene, chloroform and hexone and 0.1M $NaClO_4$ have also been determined.

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The electronic spectra of organic diselenides

By GÖRAN BERGSON

With 1 figure in the text

The spectrochemical properties of organic selenium compounds have been very little investigated. An extensive knowledge of the spectra of such compounds would, however, be of great theoretical importance because by the comparison of these spectra with those of oxygen and particularly sulfur compounds, considerable information regarding the molecular structure and chemical reactivity of the organic compounds of the group 6 elements would be gained. Such a comparative study is in progress at this institute. The present author has investigated the ultraviolet light absorption of a large number of non-cyclic diselenides.

This communication deals only with the main results of this investigation. A complete report of the ultraviolet absorption data for the non-cyclic diselenides will be published later, in connection with an infrared spectroscopic examination of these substances. A knowledge of the absorption spectra of 1,2-diselenacyclopentane (-hexane, -heptane) and their substitution derivatives would be very important, because the corresponding sulfur compounds have recently attracted considerable interest. An investigation of these cyclic diselenides is being prepared.

It is known that the non-cyclic disulfides show one characteristic selective absorption in the vacuum ultraviolet region with a maximum at about $200\text{ m}\mu$ and one absorption maximum at $250\text{ m}\mu$ in the near ultraviolet region (1). KOCH (2) has, however, shown that this is not a rule without exceptions; he observes that di-*tert.*-butyldisulfide is not able to produce the characteristic $250\text{ m}\mu$ absorption maximum, but only gives a continuous absorption with increasing extinction towards shorter wavelengths. Furthermore, SCHOTTE (3) has recently shown that disulfides which have a carboxylic group linked to the carbon atom which forms the C-S link, behave in the same abnormal manner as di-*tert.*-butyldisulfide. In order to get an idea of the problem which is set up by the occurrence of these effects it is essential to visualize the conformation of the C-S-S-C group. The bonds formed by the sulfur atoms involve mainly $3p$ -orbitals, and each sulfur atom has in addition two unshared pairs of outer electrons. One of these pairs occupies a p -orbital and the other an s -orbital. PAULING (4) has shown that the dihedral angle between the planes C-S and S-S-C is determined principally by the repulsion of these non-bonding p -electrons. The normal value of the dihedral angle is found to be about 100° . The value of the C-S-S bond angle is 105° . In the stable S_8 molecule the dihedral angle is found to be 102° , close to the normal value, which minimizes the energy of the X-S-S-X group. By a similar consideration of the normal state of selenium, PAULING concludes that

the Se-Se-Se bond angle is 105° and the dihedral angle 102° . Thus, we can establish that the energetically most stabilized state of both the C-S-S-C and the C-Se-Se-C group constitutes a skew conformation, with a bond angle of 105° , and a dihedral angle of about 100° . The only thing that makes disulfides and diselenides dissimilar, from the structural point of view, is the difference in size between the sulfur atom and the selenium atom; according to PAULING (5), the normal covalent bond radius for the two elements is 1.04 Å and 1.17 Å respectively. Recently the value 1.22 Å for the covalent radius of selenium has been reported (6).

Now, KOCH suggests that the anomalous spectroscopic behaviour of di-*tert.*-butyldisulfide is due to the fact that in this disulfide the normal value, 100° , of the dihedral angle is not fulfilled because there is steric interaction between the *tert.*-butyl groups. That there really is a distortion of the ordinary skew conformation in di-*tert.*-butyldisulfide has been confirmed by dipole-moment measurements of ROGERS and CAMPBELL (7). They find a value of the dihedral angle in di-*tert.*-butyldisulfide which deviates 10° from the normal value. KOCH's hypothesis is also supported by the fact, mentioned above, that a carboxylic group causes the same effect as the three methyl groups attached to the same carbon atom. These groups have entirely different electronic character, and therefore one might perhaps believe that the spectral anomalies of di-*tert.*-butyldisulfide and e.g. dithiodiglycolic acid are not caused by an electronic effect. However, we do not know the dihedral angle of any carboxylic substituted disulfide. The question whether there are steric or electronic effects which cause the abnormal light absorption properties of these compounds can therefore scarcely be regarded as settled by KOCH's arguments, as has already been clearly pointed out by SCHOTTE (3).

If we consider the structural similarities of the C-S-S-C and C-Se-Se-C groups mentioned above, and the difference in size between the sulfur atom and the selenium atom, it is evident that a study of the electronic spectra of diselenides would throw some new light on the problem. Substituents attached to the Se-Se group are more distant from each other than if they were attached to an S-S group. The spatial interaction of the substituents in a diselenide must therefore be smaller than in a disulfide. This is confirmed by the dipole-moment measurements mentioned above (7). The deviation in dihedral angle for di-*tert.*-butyldisulfide was found to be 10° from the normal value, whereas the dihedral angle of di-*tert.*-butyldiselenide differs from that in diethyldiselenide by only 1.5° , which is within the limits of experimental error. If, now, the spectral properties of disulfides and diselenides were a simple function of the dihedral angle, then the electronic spectrum of di-*tert.*-butyldiselenide would only show very small differences from that of a normal diselenide, and the same would, in some degree, be true for a diseleniumdicarboxylic acid such as diselenium-diglycolic acid. The experimental results of the present investigation are given in Fig. 1. The ultraviolet absorption of three diselenides namely di-*n*-butyldiselenide, di-*tert.*-butyldiselenide and diselenium-diglycolic acid (dimethyldiselenide-dicarboxylic acid) in alcoholic solution are shown. The di-*n*-butyldiselenide can be taken as a representative for a normal diselenide. It can be seen that it exhibits a strong absorption at the long wavelength edge of the vacuum-ultraviolet region, probably corresponding to the $200\text{ m}\mu$ absorption for the disulfides. Furthermore, the $250\text{ m}\mu$ absorption of the disulfides has its correspondence in an absorption maximum at $312\text{ m}\mu$ for the diselenides. This absorption band is responsible for the yellow colour of the diselenides. From the electronic spectrum of di-*tert.*-butyldiselenide, it can be established that the characteristic $312\text{ m}\mu$ absorption band has undergone a hypsochromic

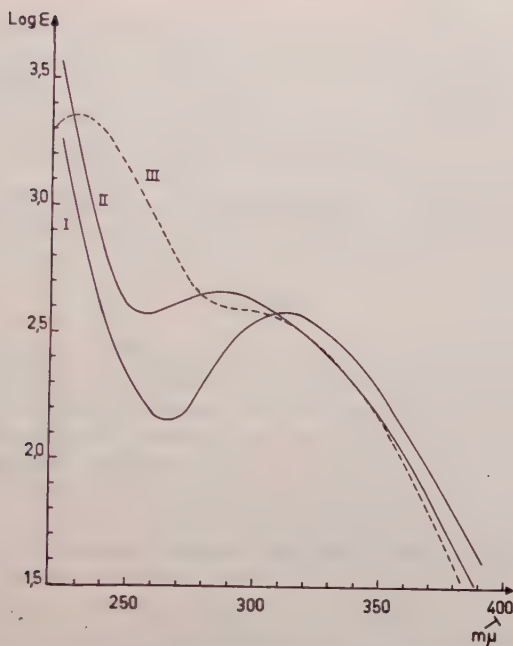


Fig. 1. Ultraviolet absorption of I, di-*n*-butyldiselenide; II, di-*tert.*-butyldiselenide; III, diseleniumdiglycolic acid.

shift to the extent of 26 $m\mu$, so that its maximum now lies at 286 $m\mu$. The peculiar fact that the 250 $m\mu$ disulfide absorption peak totally disappears in the case of di-*tert.*-butyldisulfide can now be easily explained. (Cf. also SCHOTTE's works on carboxylic substituted disulfides.) For if the disulfide absorption band undergoes a hypsochromic shift of the same order of magnitude as the diselenide band in the *tert.*-alkyl compound, the absorption bands of di-*tert.*-butyldisulfide would be so close together that the resulting absorption would be continuous at least above 220 $m\mu$, where the examination begins. A beautiful example of such an overlap of electronic bands can be seen in the absorption curve for diselenium-diglycolic acid (Fig. 1). Here the higher frequency diselenide band has undergone a bathochromic shift so that we can see an absorption peak at 229 $m\mu$. At the same time, the characteristic 312 $m\mu$ band has shifted towards shorter wavelengths. In the resulting absorption curve the longer wavelength maximum now appears only as an inflexion at 293 $m\mu$. Only a slight additional approach of the bands would have caused a totally continuous absorption. These facts give a phenomenological explanation of the spectroscopic behaviour of di-*tert.*-butyldisulfide.

We can now return to the primary question as to whether or not it is the spatial interaction of the substituents and the following change in dihedral angle which causes the spectral anomalies of di-*tert.*-butyldisulfide and dithiodiglycolic acid. It was shown that even in di-*tert.*-butyldiselenide the normal absorption maximum shifted 26 $m\mu$. Now, the deviation in dihedral angle from the normal value was 10° for di-*tert.*-butyldisulfide and about 1.5° for di-*tert.*-butyldiselenide. If these deviations could be related to the hypsochromic shifts, this shift would, for the disulfide, be much larger than would be reasonably expected. The same reasoning must in prin-

ciple hold for the carboxylic substituted compounds. Thus we can establish that the hypothesis of spectral properties as a *simple* function of dihedral angle cannot hold. If we want to take a step nearer the nature of the problem, we can also say that the changes in electronic distribution of the C-S-S-C and C-Se-Se-C groups which are responsible for the change in excitation energy are not *solely* caused by any spatial interaction of the substituents.

The principal causes of the spectral peculiarities of the disulfides and diselenides are surely to be found in the electronic properties of the substituents. The problem is certainly a rather complex one, and no accurate solution can be given at present. Some new aspects which may explain a few of the features of the spectra in Fig. 1 will however be given.

In a normal diselenide the σ -orbitals of the C-H bonds can probably hyperconjugate with the orbitals of the selenium atom. In the di-*tert.*-butyldiselenide the hyperconjugation is not possible, because the C-C σ -orbitals overlap less than the C-H σ -orbitals. It is a well-known fact that, for quantum mechanical reasons, when a molecule becomes conjugated, the energy of the excited state is lowered more than the energy of the ground state, so that the conjugated molecule shows a bathochromic effect if compared with the unconjugated molecule. It is therefore not surprising that di-*n.*-butyldiselenide absorbs at longer wavelengths than di-*tert.*-butyldiselenide. A study of the absorption of dimethyldiselenide and di-*iso*-propyldiselenide in addition to those already studied might throw further light on this point. A conjugation effect cannot, however, explain the hypsochromic shift of the 312 $m\mu$ absorption band in the carboxylic substituted compound. The effect of the carboxylic group is a withdrawal of the electrons from the C-Se-Se-C group. This will cause an increase in ionization potential because the mutual repulsion of the electrons in the C-Se-Se-C group will decrease. This increase in ionization potential will, however, be greater for the ground state than for the excited state. The result will be a shift of the absorption band towards shorter wavelengths, which is really observed for the 312 $m\mu$ band. The bathochromic shift of the higher frequency band in diselenium-diglycolic acid might be interpreted as due to conjugation between the carboxylic group and the diselenide group. For BRAUDE (8) has shown that there really is a weak interaction between chromophores and/or auxochromes which are separated by one or more saturated carbon atoms. It has also been found that the effect disappears when there are two CH_2 groups between the selenium atom and the carboxylic group (9). It must be remembered that we do not know exactly which molecular orbitals really are involved in the various excitations. The above views can therefore only be regarded as a working hypothesis. Further views on the interpretations of these spectra will be given later in connection with more experimental data. From the above it is evident that the selenium compounds are more suitable for a theoretical study than the disulfides, because their absorption lies at longer wavelengths and the two absorption bands are more separate.

Experimental

A Beckman model DU photoelectric quartz spectrophotometer equipped with a hydrogen discharge lamp was used for the ultraviolet absorption measurements. All substances were studied in 96% alcoholic solution at room temperature. The error in wavelength can be taken as $\pm 1 m\mu$, and that in the extinction as a few per cent.

The diselenides used in the investigation

Di-n-butyldiselenide: This compound was prepared according to STONER and WILLIAMS (10). B.p. 123–125°/13. $n_D^{25} = 1.5373$.

Di-tert.-butyldiselenide: This compound was prepared from *tert.*-butylmagnesium-chloride and selenium. Cf. ROGERS and CAMPBELL (7). B.p. 102–104°/19. $n_D^{25} = 1.5332$.

Diseleniumdiglycolic acid: M.p. 98–100°. Anal.: Subst.: 0.2951; 0.3327 g; 15.70; 17.73 ml of 0.1360N NaOH. Equ. wt. calc. 138.0. Found: 138.2; 138.0.

Summary

The electronic spectra of di-*n*-butyldiselenide, di-*tert.*-butyldiselenide and diseleniumdiglycolic acid in the region 220–400 m μ have been studied.

These spectra have been compared with those of the corresponding sulfur compounds.

A theoretical discussion of the spectra has been given, which has resulted in some new aspects on the light absorption mechanism of these substances.

The author wishes to express his gratitude to Professor ARNE FREDGA for many valuable discussions, and for putting the diseleniumdiglycolic acid at his disposal. The author is also indebted to Dr. LENNART SCHOTTE for interesting discussions.

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Tryckt den 11 november 1955

Uppsala 1955. Almqvist & Wiksells Boktryckeri AB



On mercapthiadiazines and *N*-aminothiazolethiones

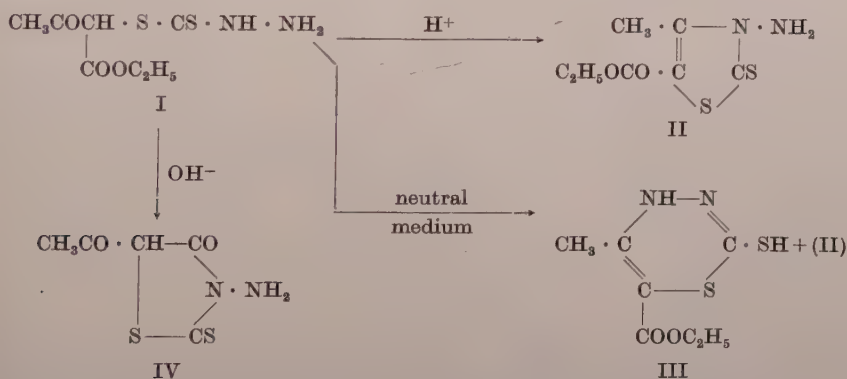
Part II¹

By JAN SANDSTRÖM

With 3 figures in the text

1. Introduction and survey

For an investigation of the reactions between dithiocarbhydrazides and ethyl α -chloroacetoacetate (1) it was necessary to prepare the ethyl 2-mercapto-5-methyl-1,3,4,4H-thiadiazine-6-carboxylate (III). This compound has previously been described by MATHES (2), who obtained it from ammonium dithiocarbazate and ethyl α -chloroacetoacetate. However, his method gives a rather poor yield, and the product is difficult to isolate from the extensive amounts of by-products. These by-products may be formed by reaction between ethyl α -chloroacetoacetate and the intermediate [1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide (I). The side-reactions are suppressed if an excess of ammonium dithiocarbazate is present during the reaction. In this way the intermediate dithiocarbhydrazide (I) is obtained in good yield, m.p. 109°–110°. This dithiocarbhydrazide reacts with benzaldehyde to give a hydrazone, m.p. 108°–109°, and it can eliminate water and cyclise in two different ways.



¹ Part I: see ref. (4).

When the hydrazide is heated in an ethanolic hydrochloric acid solution, it gives ethyl 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylate (II), m.p. 76°–77°. The presence of a free amino group in (II) is shown by the reaction with benzaldehyde, in which a Schiff base is formed, m.p. 120°–121°, and by the reaction with nitrous acid, which gives the previously (3) described bis-[4-methyl-5-carbethoxy-thiazol-2-yl] disulphide, m.p. 122–123°.

The ester group in (II) is easily hydrolysed by alkali to give the corresponding carboxylic acid, m.p. 204°–205°, and this, on heating above its melting point, is decarboxylated in low yield to the previously (4) described 3-amino-4-methyl-thiazole-2(3)-thione. The acid also reacts with benzaldehyde to give a Schiff base, m.p. 209°–210°.

When the dithiocarbhydrazide (I) is heated in aqueous ethanol, it eliminates water and gives about 20 % of (II) and 70–80 % of (III). The alkali-sensitivity of (III) excludes the use of aqueous sodium hydroxide for the separation of the two products, and for the same reason an attempted separation by chromatography on alumina was unsuccessful. The separation can be effected by recrystallization from carbon tetrachloride. Most of the mercaptothiadiazine (III) first separates in a fairly pure state, and from the mother liquor the aminothiazolethione (II) is obtained after extraction of the remaining mercaptothiadiazine with aqueous sodium hydroxide.

The course of the dehydration of (I) is probably a nucleophilic attack by either of the nitrogen atoms on the carbonyl group, followed by elimination of water to give the final products. The nucleophilic attack is catalyzed by acids, but in acid medium the 1-amino group is inactivated by the addition of a proton, which accounts for the rapid and exclusive formation of the aminothiazolethione (II) under these circumstances. In neutral medium the more nucleophilic 1-nitrogen atom may react faster than the 2-nitrogen atom, which is part of a thioamide group, and hence the formation of the mercaptothiadiazine (III) takes precedence.

Like the previously (4) described mercaptodiazines, (III) is converted to the isomeric aminothiazolethione (II), when heated in acid ethanolic solution. Probably the dithiocarbhydrazide (I) is an intermediate.

When the dithiocarbhydrazide (I) is dissolved in *N*-NaOH and the solution is acidified with hydrochloric acid, ethanol is eliminated and 3-amino-5-acetyl-rhodanine (IV) is formed, m.p. 140°–141°. It gives a Schiff base with benzaldehyde, m.p. 174°–175°, and a 2,4-dinitrophenylhydrazone, m.p. 206°–207°. The formation of the aminorhodanine (IV) could occur in two ways. Either the cyclisation occurs directly under the influence of alkali, or the ester group of (I) is first hydrolysed to the salt of the corresponding carboxylic acid, which then, like the carboxymethyldithiocarbhydrazide (5), cyclises on acidification. The first way is strongly supported by a comparison of the ultra-violet absorption spectra of (I) and (IV) as shown in Diagram 1. This idea is also supported by the reaction of a solution of (I) in alkali with benzaldehyde. On acidification, the previously mentioned benzaldehyde Schiff base of (IV) is obtained, whereas, if the salt of the carboxylic acid corresponding to (I) had been an intermediate, the benzaldehyde hydrazone of this acid could have been expected as product.

When methyl iodide is added to an alkaline solution of the amino-acetyl-rhodanine (IV), a methyl derivative of (IV) is formed, m.p. 199°–200°. It forms a Schiff base with benzaldehyde, m.p. 183°–184°, which is also obtained when the benzaldehyde Schiff base of (IV) reacts with methyl iodide in alkaline solution. In the same way benzyl chloride reacts with (IV) to give a benzyl derivative, m.p. 193°–194°, benzalde-

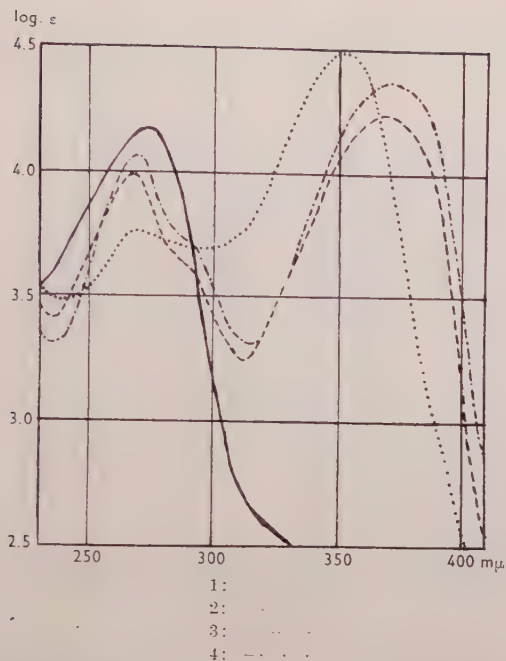
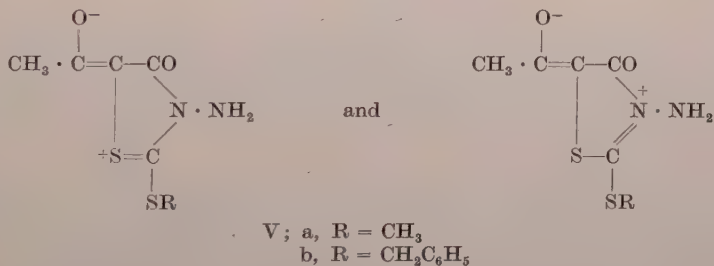


Diagram 1.—1. I, $c = 10^{-4}$ in ethanol. 2. I, dissolved in 2 equivalents of N -NaOH and diluted to $c = 4 \times 10^{-5}$ with ethanol. 3. IV, $c = 4 \times 10^{-5}$ in 0.01 N ethanolic HCl. 4. IV, dissolved in 2 equivalents of N -NaOH and diluted to $c = 4 \times 10^{-5}$ with ethanol.

hyde Schiff base m.p. 192° – 193° . Both the methyl and the benzyl derivative of (IV) fail to react with 2,4-dinitrophenylhydrazine and with hydroxylamine in pyridine, and when they are heated in acid or alkaline solution, they are decomposed with formation of methyl and benzyl mercaptan respectively. The lack of carbonyl reactivity and the fact that the new substituents are attached to sulphur can only be explained by meso-ionic structures for the two compounds. They are probably best described as resonans hybrides of the many possible polar structures such as



According to the Editor of J. Chem. Soc. (6) the name of (Va) is 3-amino-5-1'-hydroxyethylidene-2-methylthio-4-oxothiazolinium hydroxide anhydro-salt. As could have been expected, both (Va) and (Vb) are appreciably soluble in water.

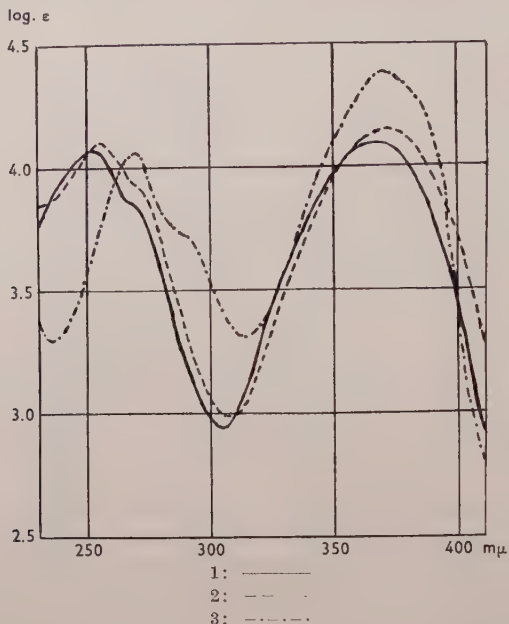
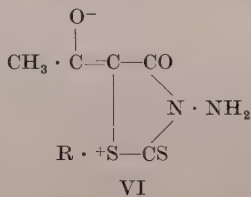
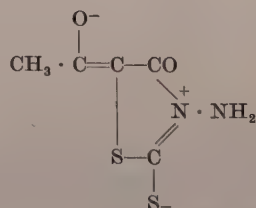
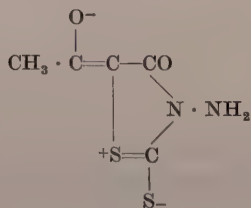


Diagram 2.—1. Va, $c = 4 \times 10^{-5}$ in ethanol. 2. Vb, $c = 4 \times 10^{-5}$ in ethanol. 3. IV, $c = 4 \times 10^{-5}$ in 8×10^{-4} N ethanolic NaOH.

The available data do not definitely exclude the alternative formula (VI), but this is regarded as less probable for the following reasons. The structures (V) and the anion of (IV) have rather similar possibilities for resonance, whereas these possibilities



in (VI) are rather different. The ultra-violet absorption spectra of (IV) in alkaline solution and of its methyl and benzyl derivatives are of the same general type (Diagram 2), which would not have been expected if the structure of the derivatives had been according to (VI).



IV-anion

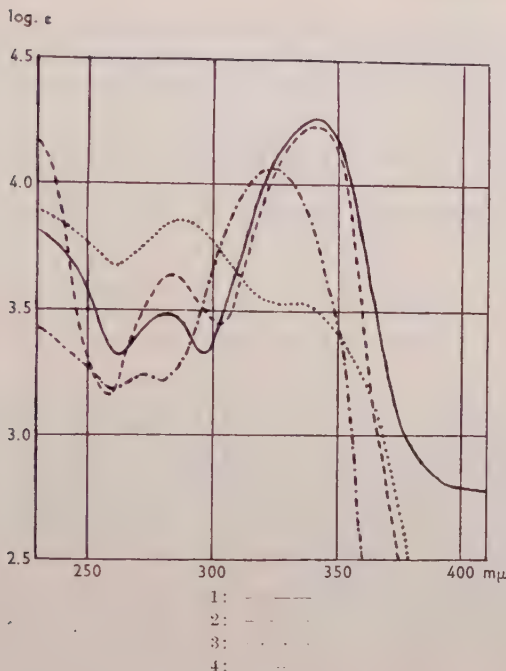
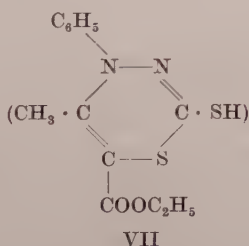


Diagram 3.—1. II, $c = 5.0 \times 10^{-5}$ in ethanol and in 10^{-3} $N\text{-NaOC}_2\text{H}_5$. 2. X, $c = 5.0 \times 10^{-5}$ in ethanol and in 10^{-3} $N\text{-NaOC}_2\text{H}_5$. 3. III, $c = 5.0 \times 10^{-5}$ in ethanol. 4. III, $c = 5.0 \times 10^{-5}$ in 10^{-3} $N\text{-NaOC}_2\text{H}_5$.

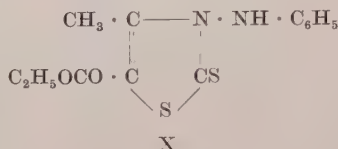
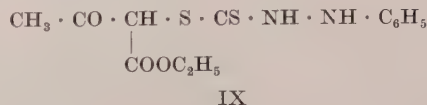
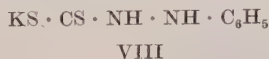
Furthermore, a compound of formula (VI) may be expected to give rise to a thioether rather than to a mercaptan on alkaline decomposition.

In the reaction between the amino-rhodanine (IV) and nitrous acid, no definable product is obtained.



Attempts were also made to prepare the ethyl 2-mercapto-4-phenyl-5-methyl-1,3,4,4H-thiadiazine-6-carboxylate (VII). Ethyl α -chloroacetoacetate reacts with potassium 1-phenyldithiocarbazate (VIII) to give 1-phenyl-[1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide (IX), m.p. $129^\circ\text{--}130^\circ$. When this dithiocarbhydrazide is heated in acid ethanolic solution, water is eliminated and a crystalline product is formed, m.p. $78^\circ\text{--}79^\circ$. This product is not soluble in aqueous alkali, contrary to what should have been expected for the mercapthothiadiazine (VII), and a comparison of its ultra-violet absorption spectrum with those of ethyl 3-amino-4-methyl-thiazole-

2(3)-thione-5-carboxylate (II) and ethyl 2-mercapto-5-methyl-1,3,4,4H-thiadiazine-6-carboxylate (III) makes it rather probable that the new compound is ethyl 3-anilino-4-methyl-thiazole-2(3)-thione-5-carboxylate (X).



The absorption of (X) can as a first approximation be regarded as the sum of the absorption of (II) and that of aniline, since the thiazolethione group and the anilino group in (X) are isolated chromophores. Aniline has its long-wavelength absorption maximum at 280 m μ with $\epsilon = 1430$ (7), which may account for the higher extinction value of (X) in this region.

When the dithiocarbhydrazide (IX) is heated in aqueous-ethanolic solution, it gives a low yield of (X) together with alkali-soluble, non-crystalline products. When (IX) is dissolved in alkali, and the solution is acidified, only non-crystalline products are obtained.

The ester (X) can be hydrolysed to the corresponding carboxylic acid, m.p. 185°–186°.

2. Experimental

A. [1'-Carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide

A solution of 16.5 g of ethyl α -chloroacetoacetate in 150 ml of ethanol was added dropwise with cooling and stirring to a solution of 25 g of ammonium dithiocarbamate in 150 ml of water. A clear, yellow solution resulted, from which crystals soon began to separate. On the following day 12.1 g of colourless, rodshaped prisms had separated, m.p. 103°–105°. The filtrate was evaporated to about half the volume, when 6.8 g of pale yellow prisms had separated, melting between 85° and 100°. The latter product was recrystallized from a mixture of 10 ml of chloroform and 10 ml of carbon tetrachloride and gave 5.0 g of colourless, rodshaped prisms, m.p. 103°–105° alone and also on admixture with the product first obtained. The two crops were combined and were recrystallized from a mixture of 25 ml of chloroform and 25 ml of carbon tetrachloride. The yield was 15.0 g of colourless, rodshaped prisms, m.p. 109°–110°, according to analyses consisting of [1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide (I).

For $\text{C}_7\text{H}_{12}\text{O}_3\text{N}_2\text{S}_2$ (236.31).	calc.:	C 35.6	H 5.12	N 11.86	S 27.1 %,
	found:	C 35.7* ¹	H 5.29*	N 11.93	S 27.2* %.

¹ The analyses marked with an asterisk have been performed by Miss M. WESTERDAHL at the Analytical Department, University of Lund, and the rest, with the exceptions mentioned later, by the author.

The mother liquor from the first recrystallization of the second crop of the hydrazide (I) was evaporated and gave a residue of 1.8 g of pale yellow flakes, melting between 70° and 90°. The product was extracted with 15 ml of hot ethanol and gave a residue of 0.4 g of pale yellow prisms, m.p. 119°–120° alone and also on admixture with pure sulphur. This sulphur probably results from air oxidation of the excess ammonium dithiocarbazate. The ethanolic solution on the addition of 15 ml of water became turbid and deposited 1.0 g of pale yellow flakes, m.p. 97°–98°, which according to analyses consisted of ethyl 2-mercapto-5-methyl-1,3,4,4H-thia-diazine-6-carboxylate (III).

For $C_7H_{10}O_2N_2S_2$ (218.29) calc.: C 38.5 H 4.61 %,
found: C 38.7* H 4.77* %.

For this compound MATHES (2) reports m.p. 97°–98°.

A solution of 0.3 g of benzaldehyde in 5 ml of ethanol was added to a solution of 0.6 g of the dithiocarbhydrazide (I) in 5 ml of ethanol. The clear, colourless mixture remained unaltered to the next day, when 10 ml of water was added. An oil separated, which presently solidified to 0.8 g of a colourless crystal mass, m.p. 107°–108°. The product was recrystallized from 40 ml of ligroin (b.p. 80°–100°) and gave 0.6 g of colourless, felted hairs, m.p. 108°–109°. According to analyses the product was benzaldehyde [1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide.

For $C_{14}H_{16}O_3N_2S_2$ (324.41) calc.: C 51.8 H 4.79 N 8.64 S 19.77 %,
found: C 51.9* H 4.96* N 8.65 S 19.92* %.

B. The anhydrides of [1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide

To a solution of 2.4 g of the dithiocarbhydrazide (I) in 10 ml of ethanol were added two drops of concentrated hydrochloric acid. The mixture slowly turned yellow, and on the following day the solvent was evaporated and gave a residue of 2.2 g of pale yellow crystals, melting between 65° and 72°. The product was extracted with 10 ml of *N*-NaOH, and 2.0 g of colourless crystals remained undissolved, m.p. 75°–76°. Recrystallization from 10 ml of ethanol afforded 1.6 g of colourless, rhombic prisms, m.p. 76°–77°. According to analyses it was ethyl 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylate (II).

For $C_7H_{10}O_2N_2S_2$ (218.29) calc.: C 38.5 H 4.61 N 12.84 S 29.4 %,
found: C 38.5* H 4.68* N 12.83 S 29.6* %.

A solution of 0.6 g of the ester and 0.3 g of benzaldehyde in 5 ml of ethanol remained clear and colourless for one day, but on the addition of two drops of concentrated hydrochloric acid it rapidly solidified to a cake of yellow, felted hairs, m.p. 120°–121°. The product, 0.8 g, was recrystallized from a mixture of 3 ml of benzene and 10 ml of absolute ethanol and gave 0.7 g of yellow, felted hairs of unchanged melting point. According to analyses it consisted of the benzaldehyde Schiff base of the ethyl 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylate.

For $C_{14}H_{14}O_2N_2S_2$ (306.39) calc.: C 54.9 H 4.61 N 9.14 S 20.9 %,
found: C 54.7* H 4.47* N 9.10 S 20.9* %.

The amino-thiazole-thione (II), 1.1 g, was ground with 10 ml of glacial acetic acid, and 1.0 g of solid sodium nitrite was added in portions. The mixture was brown and clear, but within a minute it solidified to a porridge of crystals, and the colour rapidly faded. The yield was 0.9 g of a colourless, crystalline powder, m.p. 119°–121°. Recrystallization from 15 ml of ethanol gave 0.8 g of colourless, rodshaped prisms, m.p. 122°–123°, according to analyses consisting of bis-[4-methyl-5-carbethoxy-thiazol-2-yl] disulphide.

For $C_{14}H_{16}O_4N_2S_4$ (404.53) calc.: C 41.6 H 3.99 %,
found: C 41.9*** H 4.22** %.

This compound has previously been described by D'AMICO (3), who reports m.p. 120°–121°.

A solution of 4.4 g of the ester (II) in 50 ml of ethanol and 30 ml of *N*-NaOH was refluxed for twenty minutes. After cooling, the mixture was acidified with 6 ml of 5 *N*-HCl and deposited 3.5 g of colourless flakes, m.p. 200°–201° (decomp.). The product was recrystallized from 35 ml of ethanol and gave 2.5 g of colourless flakes, m.p. 203°–204° (decomp.). According to analyses it was 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylic acid.

For $C_5H_6O_2N_2S_2$ (190.24) calc.: C 31.6 H 3.18 N 14.73 S 33.7 %, equiv. w.: 190.2,
found: C 31.5* H 3.17* N 14.78 S 33.7* %, equiv. w.: 189.3.

Benzaldehyde, 0.3 g, was added to a hot solution of 0.5 g of this acid in 5 ml of glacial acetic acid. On cooling, the mixture deposited 0.6 g of yellow, rodshaped prisms, m.p. 204°–205° (decomp.). Recrystallization from 20 ml of glacial acetic acid afforded 0.4 g of yellow, oblique plates, m.p. 209°–210° (decomp.), consisting according to analyses of the benzaldehyde Schiff base of the above mentioned carboxylic acid.

For $C_{12}H_{10}O_2N_2S_2$ (278.34) calc.: C 51.8 H 3.62 N 10.07 S 23.0 %, equiv. w.: 278.3,
found: C 51.5* H 3.61* N 10.11 S 23.3* %, equiv. w.: 280.0.

The 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylic acid, 1.0 g, was kept at 210° until the evolution of carbon dioxide had ceased. The dark brown, tarry residue was extracted with 100 ml of boiling ligroin (b.p. 80°–100°). On cooling, the solution deposited 0.2 g of colourless, rodshaped prisms, m.p. 94°–95°, undepressed on admixture with pure 3-amino-4-methyl-thiazole-2(3)-thione (4).

For $C_4H_6N_2S_2$ (146.23) calc.: N 19.16 %,
found: N 19.22 %.

The dithiocarbonylhydrazide (I), 4.8 g, was dissolved in 20 ml of hot ethanol, 20 ml of water was added, and the solution was refluxed for 30 minutes, after which the ethanol was distilled off. The mixture then turned cloudy and deposited an oil, which on cooling solidified to 4.4 g of a pale yellow crystal mass, melting between 80° and 90° after sintering from 70°. The product was recrystallized from 40 ml of

¹ The analyses marked with two asterisks have been performed by Drs. WEILER and STRAUSS (Oxford).

carbon tetrachloride and afforded 3.1 g of pale yellow, long plates, m.p. 98°–99° alone and also on admixture with the ethyl 2-mercapto-5-methyl-1,3,4,4H-thiadiazine-6-carboxylate (III) described above. Further recrystallizations from carbon tetrachloride did not change the melting point.

For $C_7H_{10}O_2N_2S_2$ (218.29) calc.: C 38.5 H 4.61 %,
found: C 38.4* H 4.67* %.

The mother liquor was evaporated to a sticky mass, which was ground with 10 ml of *N*-NaOH. A residue of 1.1 g of a pale yellow crystal mass remained undissolved, m.p. 74°–75°. Recrystallization from 5 ml of ethanol afforded 0.8 g of colourless, irregular prisms, m.p. 76°–77°, undepressed on admixture with pure ethyl 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylate (II).

Solid ethyl 2-mercapto-5-methyl-1,3,4,4H-thiadiazine-6-carboxylate (III), 1.1 g, was ground with 10 ml of *N*-NaOH. A deep red solution resulted, which soon turned cloudy. Norite was added, and the mixture was rapidly filtered and acidified with 2 ml of 5 *N*-HCl, within 5 minutes after the addition of NaOH. An emulsion resulted, which on the following day had deposited 0.5 g of brown-yellow, irregular prisms, m.p. 121°–124° (after sintering). Recrystallization from 100 ml of ligroin (b.p. 80°–100°) yielded 0.3 g of pale yellow, rodshaped prisms, m.p. 126°–127°, undepressed on admixture with authentic 2-mercapto-5-methyl-1,3,4,4H-thiadiazine (2). This result is not compatible with MATHES' observation, that the ester (III) is hydrolysed and decarboxylated by hot aqueous alkali but is unaffected by the same reagent at room temperature.

A solution of 1.1 g of the mercaptothiadiazine (III) in 10 ml of *N* ethanolic hydrochloric acid was refluxed for two hours. After evaporation of the solvent, 1.1 g of pale yellow, crystalline mass was obtained as a residue, m.p. 76°–77°, undepressed on admixture with ethyl 3-amino-4-methyl-thiazole-2(3)-thione-5-carboxylate (II). The product reacted with benzaldehyde to give a Schiff base, m.p. 120°–121°.

C. 3-Amino-5-acetyl-rhodanine

Solid [1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide (I), 2.4 g, was dissolved in 20 ml of *N*-NaOH. Then 4 ml of 5 *N*-HCl were added immediately to the pale yellow solution, and 1.8 g of pale yellow flakes separated, m.p. 136°–137° (decomp.). Recrystallization from 30 ml of toluene yielded 1.6 g of pale yellow plates, m.p. 140°–141° (decomp.), consisting according to analyses of 3-amino-5-acetyl-rhodanine (IV).

For $C_5H_6O_2N_2S_2$ (190.24) calc.: C 31.6 H 3.18 N 14.73 S 33.7 %, equiv. w.: 190.2,
found: C 31.8* H 3.18* N 14.74 S 33.5* %, equiv. w.: 190.41.

This aminorhodanine, 0.5 g, and benzaldehyde, 0.3 g, gave a clear solution in 5 ml of ethanol, but the addition of one drop of concentrated hydrochloric acid caused the separation of 0.6 g of yellow plates, m.p. 171°–173°. The product was recrystallized twice from 5 and 4 ml of ethanol and gave 0.4 g of yellow, rhombic plates, m.p.

¹ On rapid titration, sharp end point against phenolphthalein.

174°–175°. According to analyses the product was the benzaldehyde Schiff base of the aminorhodanine (IV).

For $C_{12}H_{10}O_2N_2S_2$ (278.34)	calc.:	C 51.8	H 3.62	N 10.06	S 23.0 %
	found:	C 51.8*	H 3.80*	N 10.11	S 22.9* %

The dithiocarbhydrazide (I), 0.6 g, was dissolved in 5 ml of *N*-NaOH, and a solution of 0.3 g of benzaldehyde in 5 ml of ethanol was added. After one hour the solution was acidified with 1 ml of 5 *N*-HCl, and on the following day the solution had deposited 0.7 g of pale yellow plates, m.p. 165°–168°. The product was recrystallized from 5 ml of glacial acetic acid and yielded 0.5 g of yellow plates, m.p. 174°–175°, undepressed on admixture with the benzaldehyde Schiff base of 3-amino-5-acetyl-rhodanine.

A solution of 0.49 g of amino-acetyl-rhodanine (IV) in 5 ml of ethanol was added to a hot solution of 0.47 g of 2,4-dinitrophenylhydrazine in 5 ml of 6 *N*-HCl in 50 % aqueous ethanol. On cooling, the mixture deposited 0.85 g of red-yellow, rod-shaped prisms, m.p. 206°–207° (decomp.). The product was too slightly soluble in the common solvents to be recrystallized. It was soluble with decomposition in *N*-NaOH and in pyridine. According to analyses it was fairly pure 3-amino-5-acetyl-rhodanine 2,4-dinitrophenylhydrazone.

For $C_{11}H_{10}O_5N_6S_2$ (370.36)	calc.:	C 35.7	H 2.72	N 22.7	S 17.31 %
	found:	C 35.3*	H 2.90*	N 22.8	S 17.44* %

Methyl iodide, 0.5 g, was added to a solution of 0.48 g of amino-acetyl-rhodanine (IV) in 2 ml of *N*-NaOH and 5 ml of ethanol. On the following day 0.42 g of yellow, rodshaped prisms had separated, m.p. 188°–190°. Recrystallization from 20 ml of ethanol yielded 0.30 g of yellow, rodshaped prisms, m.p. 199°–200° (decomp.). According to analyses they consisted of 3-amino-5-1'-hydroxyethylidene-2-methylthio-4-oxothiazolinium hydroxide anhydro-salt (Va).

For $C_6H_8O_2N_2S_2$ (204.26)	calc.:	C 35.3	H 3.95	N 13.72	S 31.4 %	M: 204,
	found:	C 35.2**	H 3.99**	N 13.7**	S 31.6** %	M: 211.

This compound, 0.10 g, was heated with 3 ml of *N*-NaOH, while air was led over the mixture and aspirated through a trap containing a 4 % aqueous mercuric cyanide solution. The material (Va) soon dissolved, and a copious white precipitate was formed in the trap. The yield was 0.052 g (calc. 0.074 g), m.p. 168°–171°. Recrystallization from 35 ml of ethyl acetate yielded 0.020 g of colourless, glistening plates, m.p. 176°–177°, undepressed on admixture with an authentic specimen of bis-methylthio-mercury. KLASON (8) reports m.p. 175°.

Benzaldehyde, 0.030 g, was added to a solution of 0.060 g of the salt (Va) in 3 ml of glacial acetic acid. On the following day 0.070 g of yellow hairs had separated, m.p. 180°–181°. Recrystallization from 3 ml of ethanol afforded 0.048 g of yellow hairs, m.p. 183°–184° (prod. 1).

Methyl iodide, 0.2 g, was added to a solution of 0.28 g of the benzaldehyde Schiff base of (IV) in 5 ml of ethanol and 1 ml of *N*-NaOH. On the following day no crystallization had occurred. Free evaporation yielded a somewhat sticky crystalline residue, which was recrystallized from 10 ml of ethanol and yielded 0.14 g of yellow hairs,

m.p. 183°–184°, undepressed on admixture with prod. 1. (prod. 2). According to analyses the two products consisted of the benzaldehyde Schiff base of the salt (Va).

For $C_{13}H_{12}O_2N_2S_2$ (292.37) calc.: C 53.4 H 4.14 N 9.58 S 21.9 %,
found: C 53.1**1 H 4.04**1 N 9.48**2 S 21.6**2 %.

Benzyl chloride, 0.65 g, was added to a solution of 0.96 g of amino-acetyl-rhodanine (IV) in 5 ml of *N*-NaOH and 15 ml of ethanol. On the following day 0.60 g of pale yellow flakes had separated, m.p. 188°–189° (decomp.). Recrystallization from 40 ml of ethanol afforded 0.52 g of pale yellow plates, m.p. 193°–194° (decomp.). According to analyses the product is 3-amino-5-1'-hydroxyethylidene-2-benzylthio-4-oxothiazolinium hydroxide anhydro-salt (Vb).

For $C_{12}H_{12}O_2N_2S_2$ (280.36) calc.: C 51.4 H 4.32 N 9.99 S 22.9 %, M: 280,
found: C 51.4* H 4.32* N 9.99 S 22.8* %, M: 291.

When this compound, 0.56 g, was suspended in 5 ml of 5 *N*-HCl and steam was passed through, a colourless oil with a strong smell of benzyl mercaptan appeared in the distillate. It was taken up in 15 ml of ether, and when the ether was distilled off, a residue of 0.21 g of colourless oil was obtained. From this the 1-benzylthio-2,4-dinitrobenzene and the corresponding sulphone were prepared according to Bost *et al.* (9). The m.p. were 129°–130° and 182°–183° respectively, undepressed on admixture with authentic material. The sulphide contained 11.12 % of sulphur, calc. for $C_{13}H_{10}O_4N_2S$ (290.29) 11.04 %.

A solution of 0.14 g of (Vb) and 0.06 g of benzaldehyde in 15 ml of ethanol gave a clear solution, which on the addition of one drop of concentrated hydrochloric acid deposited 0.15 g of yellow, felted hairs, m.p. 192°–193°. Recrystallization from 10 ml of ethanol afforded 0.13 g of yellow, felted hairs of unchanged m.p. (prod. 1.).

A solution of 0.6 g of the benzaldehyde Schiff base of amino-acetyl-rhodanine (IV) in 3 ml of *N*-NaOH was added to a solution of 0.35 g of benzyl chloride in 10 ml of ethanol. On the following day the mixture had deposited 0.18 g of yellow, felted hairs, m.p. 189°–190°. Recrystallization from 12 ml of ethanol afforded 0.14 g of yellow, felted hairs, m.p. 192°–193° (prod. 2), undepressed on admixture with prod. 1. According to analyses the two products consist of the benzaldehyde Schiff base of the anhydro salt (Vb).

For $C_{18}H_{16}O_2N_2S_2$ (368.46) calc.: C 61.9 H 4.38 N 7.60 S 17.40 %,
found: C 62.1*1 H 4.58*1 N 7.55*2 S 17.44*2 %.

D. 1-Phenyl-[1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide

A solution of 8.5 g (0.05 moles) of ethyl α -chloroacetoacetate in 75 ml of ethanol was added with cooling and stirring to a solution of 16.5 g (0.075 moles) of potassium 1-phenyldithiocarbazinate (VIII) (10), in 100 ml of water and 50 ml of ethanol. The mixture deposited an oil, which soon solidified to 14.5 g of a pale brown crystalline mass, m.p. 120°–123°. The product was recrystallized twice from 50 and 40 ml of toluene and gave 9.9 g of colourless, rodshaped prisms, m.p. 129°–130°. According to analyses the product was 1-phenyl-[1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide (IX).

¹ Prod. 1.

² Prod. 2.

For $C_{13}H_{16}O_3N_2S_2$ (312.50) calc.: C 50.0 H 5.16 N 8.97 S 20.5 %,
found: C 50.2* H 5.11* N 8.96 S 20.3* %.

E. The anhydride of 1-phenyl-[1'-carbethoxy-propan-2'-on-1'-yl]-dithiocarbhydrazide

A solution of 1.6 g of the dithiocarbhydrazide (IX) in 10 ml of ethanol and 1 ml of 5 N-HCl was kept at 65° for 30 minutes. On cooling, the mixture deposited 1.3 g of pale yellow, irregular prisms, m.p. 78°–79°. Recrystallization from 5 ml of ethanol afforded 0.8 g of colourless, irregular prisms of unchanged m.p.

For $C_{13}H_{14}O_2N_2S_2$ (294.38) calc.: C 53.0 H 4.79 N 9.52 S 21.8 %,
found: C 53.1* H 4.81* N 9.56 S 21.6* %.

When the mother liquor from the crude product was evaporated to dryness, a sticky residue was obtained, from which no crystalline product could be obtained.

A solution of 0.8 g of the dithiocarbhydrazide (IX) in 10 ml of 50 % aqueous ethanol was refluxed for one hour. On cooling, the mixture deposited a colourless, semisolid mass. This was decanted and rubbed with 10 ml of N-NaOH. A residue of 0.2 g of a colourless crystal powder was obtained, m.p. 75°–76° alone and 77°–78° on admixture with the anhydride just described. The alkaline solution was acidified with 2 ml of 5 N-HCl and deposited an oil, which could not be made to crystallize.

A solution of 0.6 g of the anhydride in 10 ml of ethanol and 2.5 ml of N-NaOH was refluxed for 45 minutes. Then 10 ml of water was added, and the ethanol was distilled off. After cooling, the mixture was acidified with 3 ml of N-HCl and deposited an oil, which soon solidified to 0.5 g of a colourless crystalline mass, m.p. 183°–184° (decomp.). Recrystallization from 13 ml of toluene afforded 0.4 g of colourless, irregular prisms, m.p. 185°–186° (decomp.). According to analyses the product is a carboxylic acid formed by hydrolysis of the carbethoxy group in the anhydride just described.

For $C_{11}H_{10}O_2N_2S_2$ (266.33) calc.: C 49.6 H 3.78 N 10.52 S 24.1 %, equiv. w.: 266.3,
found: C 49.4* H 3.57* N 10.47 S 24.0* %, equiv. w.: 265.3.

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Uppsala 1955. Almqvist & Wiksells Boktryckeri AB

An investigation of moisture content and enzyme activities in barley seedlings in relation to their growth rate

By GUNNAR JANSSON

With 6 figures in the text

The problem of biochemically determining the vitality or germination capacity of seeds is intimately connected to the task of finding any seed property that stands in direct relation to the ability of this seed to germinate. Properties which have been especially investigated biochemically are certain enzyme activities. The question about the relation of these activities to germinability has sometimes been answered in the negative, sometimes in the affirmative. The enzyme, which has been mostly investigated, is catalase. GRACANIN (1) found no relation between catalase activity and vitality in his work; de VILMORIN and CAZAUBON (2), however, found a high positive correlation between catalase activity and germinability in different varieties of beans. CROCKER and HARRINGTON (3) showed a positive correlation between catalase activity and respiration in "Johnson grass seeds". The same authors, however, could not find such a relation in seeds of *Amaranthus retroflexus* (3). NANDA (4) found correlation between catalase ratio, that is catalase activity of soaked seeds divided by catalase activity of dry seeds, and germination capacity. CROCKER and HARRINGTON (3) also showed that a positive correlation exists between oxidase activity and vitality in seeds, though not so strong as for catalase. The oxidase activity was measured manometrically with pyrogallol as substrate. FINK and SCHWIEGER (5) have shown that it is possible to determine the germinability of barley seeds by treating the kernels with a solution of triphenyltetrazoliumchloride. Good kernels were coloured red at certain places and this colouring was stronger when the germinating energy of the kernels was higher. In this procedure it was the dehydrogenase activity of the seeds which was responsible for the colouring. GUSTAFSSON (6) reported that by treating barley kernels with solutions of phosphoric acid, benzidine and hydrogen peroxide containing sodium chloride and finally with petroleum ether, viable kernels remained uncoloured or were coloured clear yellow, while dead kernels showed an appreciable orange colouration. This test is based on the use of benzidine to measure the decomposition of hydrogen peroxide by substances in the barley hulls.

In this investigation the following properties of barley seedlings have been examined with respect to growth rate: moisture content, activities of dehydrogenase, catalase and amylase, and respiration capacity.

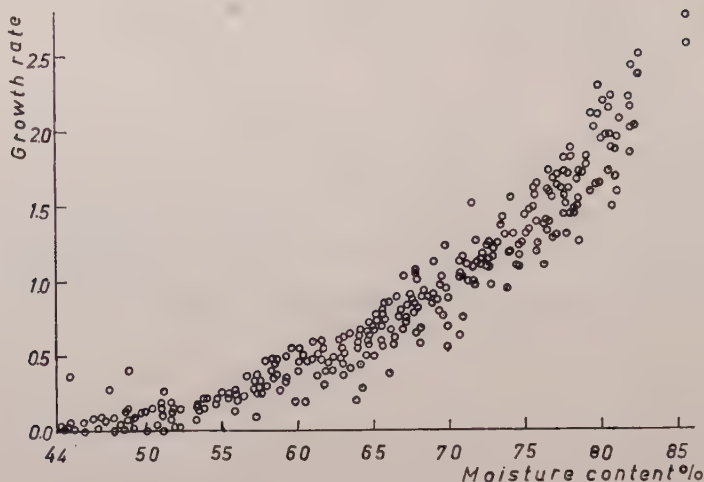


Fig. 1. Moisture content in relation to growth rate.

Experimental

All determinations were carried out on seeds which were grown at room temperature for four days in moist sand in the dark, with each of the thirteen germination experiments having the same moisture content of the sand. 25 seeds were grown in a single experiment.

For each enzyme activity experiment a series of determinations was carried out on seedlings with different growth rates. These seedlings were taken from the same germination experiment. The growth rate was defined as milligrams coleoptile formed per milligram kernel dry substance per four days. The enzyme activity was then calculated on one seedling. The connection between enzyme activity and growth rate was then calculated statistically and stated as the correlation coefficient. As a basis for calculating the enzyme activities the estimated amount of dry substance of enzyme material employed for the analysis was used. The dry weight determination was made on residual seedlings in the experiment since the whole seedling was used for the enzyme determination. It can be shown that a very high positive correlation existed between the growth rate of the seedling and its moisture content. From the curve drawn with the aid of these values the moisture contents of the seedling used for enzyme activity determinations could be calculated with rather good accuracy when the growth rates were known. The correlation coefficient for all values of moisture content in relation to growth rate which were determined for the different enzyme determinations, was calculated as 0.959. The curve in Fig. 1 of moisture content in relation to growth rate contains 300 determinations. According to this correlation coefficient there exists a very strong connection between the moisture content of a seedling and its growth rate. One explanation of this may be the fact that if a seedling has a high growth rate it has a heavy coleoptile, and because the moisture content of the coleoptile is much higher than that of the seedling as a whole, a high growth rate results in high moisture content of the seedling.

In order to establish whether this explanation is correct, one experiment was carried out in which the seedlings were grown as usual in moist sand for four days. After

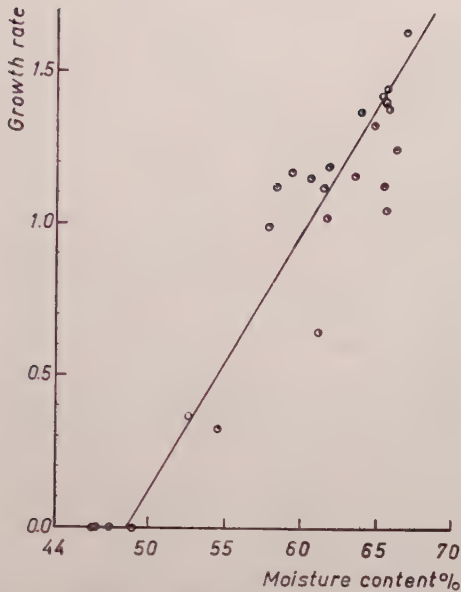


Fig. 2. Moisture content in relation to growth rate.

this time, the whole coleoptile and the roots were cut off and the moisture content of the remaining kernel was determined. The correlation coefficient between moisture content of the kernel and the growth rate of the seedling was calculated and found to be 0.942, which is of the same magnitude as before (see Fig. 2).

The dehydrogenase activity was determined by photometric measurement of the reduction of 2,6-dichlorophenolindophenol, a redox system. The activity was stated in extinction change per hour per gram dry substance of enzyme material. The value of the correlation coefficient, i.e. the strength of the connection between dehydro-

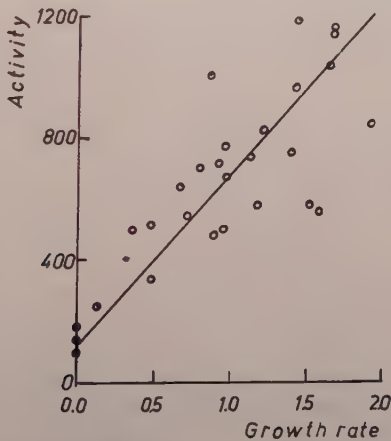


Fig. 3. Dehydrogenase activity in relation to growth rate.

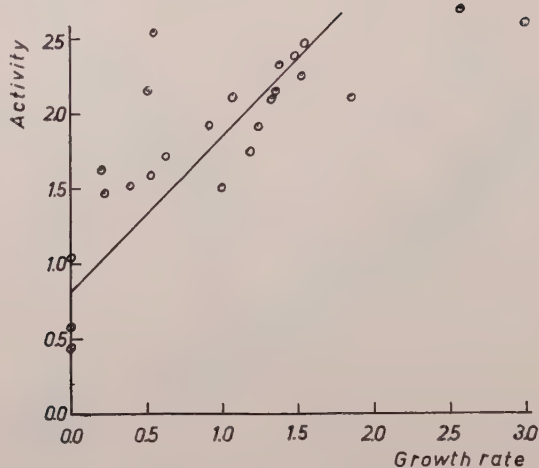


Fig. 4. Catalase activity in relation to growth rate.

genase activity and growth rate, was found to be 0.871, a high correlation between the two variables (see Fig. 3).

Catalase activity was measured according to MYRBÄCK and MYRBÄCK (7), using hydrogen peroxide as substrate. At different times the hydrogen peroxide was determined by titration with potassium permanganate. The activity was stated as the monomolecular reaction rate constant per minute per gram dry substance of enzyme material. The correlation coefficient was calculated as 0.705, a rather high correlation (see Fig. 4).

The respiration capacity was determined by measuring the oxygen uptake of the seedlings by means of a Warburg apparatus. Respiration was stated in microliters

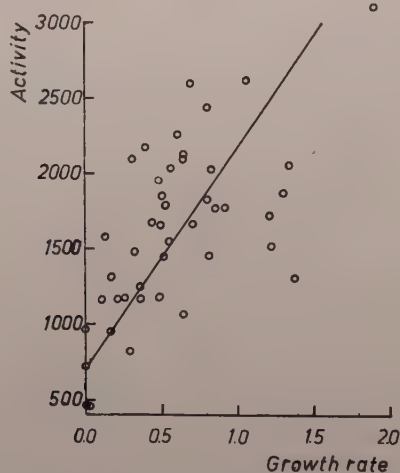
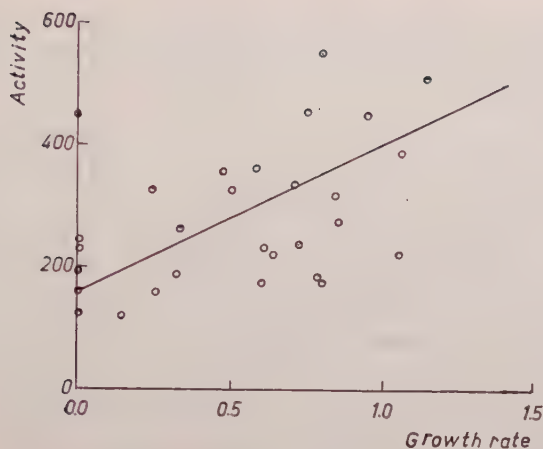


Fig. 5. Respiration activity in relation to growth rate.

Fig. 6. β -Amylase activity in relation to growth rate.

oxygen taken up per hour per gram dry substance of the seedling. The correlation coefficient was found to be 0.685, a value which is about the same as that of catalase activity (see Fig. 5).

β -Amylase activity was determined by measuring the maltose formation from starch according to MYRBÄCK and MYRBÄCK (8). The activity was calculated as milligrams maltose formed per minute per gram dry substance of enzyme material. The correlation coefficient between the two variables, enzyme activity and growth rate, was 0.424, which is much lower than those of the other enzyme activities investigated (see Fig. 6).

Table 1. Correlation coefficients for the relation between enzyme activities and growth rates.

Activity	Number of determinations	Correlation coefficients	Curve number
Moisture content in whole seedling .	300	0.959	1
Moisture content in kernel alone . . .	25	0.942	2
Dehydrogenase.	29	0.871	3
Catalase	24	0.705	4
Respiration	43	0.685	5
β -Amylase	29	0.424	6

Discussion

The purpose of this investigation has been to find any property of barley seedlings which stands in some relation to growth rate. The properties examined have been the moisture content, the respiration capacity and the enzyme activities of catalase, dehydrogenase and β -amylase. The growth rate has been defined as the weight of coleoptile formed per time unit per gram kernel dry substance. Seedlings with dif-

ferent growth rates have been investigated with respect to the properties mentioned above. The strength of the connection between growth rate on the one hand and activity on the other has been calculated statistically and stated in the form of correlation coefficient.

It has been shown that the highest positive correlation exists between moisture content of the seedling and its growth rate. The moisture content of the kernel, that is the seedling without coleoptile and roots, has a high correlation to growth rate, too. This fact may seem self-evident, because all growth must be depending on water supply. It is very interesting, however, to see how well the moisture content of the seedling and its growth rate follow each other.

The enzyme activity which has the highest positive correlation to the growth rate is that of the dehydrogenase systems. This fact is very interesting, because these enzymes are important links in the chain of enzyme reactions which break down substances of the endosperm of the seed to form others which can be used for building materials for new cells and as a source of energy for this synthesis.

Next to the correlation of the dehydrogenase activity to the growth rate stand the correlations of catalase activity and respiration power. The function of catalase, or at least one function, is to destroy the hydrogen peroxide which is formed in the cells. With a higher growth rate, it follows that more hydrogen peroxide is formed, and therefore the activity of catalase should increase in such cases.

In order to determine respiration rate, one measures the oxygen which the seedling uses for its living processes. Some of this oxygen finally reacts with the hydrogen which the dehydrogenase systems "liberate" from their substrates. In respiration, oxygen is also consumed by oxidases other than those belonging to the cytochrome system. This might be an explanation for the fact that the oxygen consumption does not show the same high correlation to growth rate as does the dehydrogenase activity. Except for the dehydrogenases the enzyme systems which use oxygen need not necessarily have a high correlation with growth rate.

The experiments carried out in this investigation show that it is not possible to point out one enzyme system whose activity has a complete correlation to growth rate. So much may be said, however, that certain systems, especially the dehydrogenases, cannot have too wide variations in their activities without the growth rate suffering therefrom. One system which, according to this investigation, can vary its activity within rather wide limits, is the β -amylase.

Summary

1. This investigation was undertaken to find out if there is any property of barley seedlings which can be said to have a higher correlation to growth rate than other properties examined.

2. The water-absorbing capacity was found to show a very high correlation to growth rate.

3. Of the enzyme activities investigated, the dehydrogenase activity showed the strongest correlation to growth rate, β -amylase showed the weakest, and catalase and respiration activities were between these two activities.

4. The proportions between the correlation coefficients were the following: Moisture content $\gg$ dehydrogenase $>$ catalase $\approx$ respiration $\gg$ β -amylase.

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Oxydo-reduktive Funktionen der aci-Reduktone. II

Von HANS VON EULER und HANS HASSELQUIST

Mit 5 Figuren im Text

Ob die mannigfachen biologischen Wirkungen der L-Ascorbinsäure (AS) auf ihre oxydo-reduktive Teilnahme an zentralen Stoffwechselreaktionen, also auf eine gemeinsame Ursache zurückgeführt werden können, ist ein biochemisches Problem von allgemeiner und wesentlicher Bedeutung.

Die leicht eintretende Oxydation der Ascorbinsäure zu Dehydro-ascorbinsäure ist, wie bekannt, reversibel, der Laktanring der Ascorbinsäure bleibt in dieser Oxydationsphase erhalten. Die darauf folgende irreversible Oxydation der Dehydro-ascorbinsäure (DHA), die teils zu Diketogulonsäure, teils zu anderen noch wenig erforschten Produkten führt, hängt in erster Linie von der Natur der angewandten Oxydationsmittel ab. Als solche kommen in Betracht:

Molekularer Sauerstoff (in Gegenwart eines Metall-Aktivators), Jod, Farbstoffe wie Dichlorphenol-indophenol (Tillmans-Reagens, TR), Hydroperoxyd (in Gegenwart oder Abwesenheit von Cuprisalzen), ferner Kaliumjodat sowie Salze anderer Persäuren.

Die hier mitzuteilenden Versuche betreffen die Wirkung dieser Oxydationsmittel in Systemen, in denen ein hochmolekularer Stoff, wie Pektin, eine oxydative Veränderung erfährt, die durch Viskositätsmessungen verfolgt wurde. (Methodik siehe dieses Arkiv 8, nr. 37.)

In vorhergegangenen Mitteilungen^{1, 2} wurde in Bestätigung früherer Arbeiten (HENGLEIN³, KERTESZ⁴, und DEUEL⁵) angegeben, dass keineswegs alle Reduktone sondern nur die aci-Reduktone die Viskosität der Pektinlösungen erniedrigen, und dass die Dehydro-ascorbinsäure, in welcher die Endiolgruppe durch eine Di-keto-gruppe ersetzt ist, die Viskosität der Pektinlösungen nur unbedeutend erniedrigt. In weiteren Versuchen war festgestellt worden:

1) Durch 3,4-Dioxy-5-methyl-tetron wird die Viskosität besonders stark erniedrigt.

¹ EULER u. M. L. STEIN, Makromol. Chemie 15, 60 (1955).

² EULER u. H. HASSELQUIST, Arkiv Kemi 8, nr 37 (1955).

³ F. A. HENGLEIN u. VOLLMERT, Makromol. Chem. 2, 77 (1948). — F. A. HENGLEIN u. J. KROHN, Makromol. Chem. 4, 308 (1950). — F. A. HENGLEIN, Moderne Methoden d. Pflanzenanalyse II, 226 (1955).

⁴ Z. J. KERTESZ, The Pectic Substances, New York, Inter. Publ. (1951).

⁵ H. DEUEL, J. SOLMS u. H. ALTERMATT, Viertelj. Schr. Naturf. Ges. Zürich 98, 49 (1953). — H. DEUEL, Helv. Chim. Acta 33, 10 (1950).

2) In Pektinlösungen, welche neben Ascorbinsäure noch Hydroperoxyd enthalten, wird, wie Titration mit TR zeigt, die Ascorbinsäure unter gleichzeitiger starker Abnahme der Viscosität schnell verbraucht.

Versuche

Verhalten der Pektine zu Alkali

Pektine sind hochmolekulare, weitgehend methoxylierte Polygalakturonsäuren (Pektinsäuren); sie werden in saurer wie in alkalischer Lösung gespalten bzw. hydrolysiert. Der ausserordentlich grosse Einfluss sehr kleiner Alkali-Konzentrationen auf die Viscosität von Pektinlösungen¹, worüber bisher keine Versuche vorlagen, wird durch folgende Angaben gekennzeichnet:

a) 4 ml Lösung von 4,0 mg Pektin/ml wurden mit 4 ml 0,1-*n* NaOH versetzt; nach 10 Min. (20°) wurde die Reaktionslösung mit 0,1-*n* HCl zurücktitriert. Verbrauch 2,53 ml; das Pektin hat somit 1,47 ml NaOH-Lös. verbraucht.

b) 4 ml Pektinlösung wie oben mit 4 ml NaOH Lös. Steht bei 20° 3 Tage und wird dann mit 0,1-*n* HCl zurücktitriert. Angewandt 1,08 ml 0,1-*n* HCl. Verbrauch durch das Pektin somit 2,92 ml NaOH.

Beim Versuch a) trat während der 10 Min. langen Alkali-Behandlung keine Viscositäts-Änderung ein. Wurde die Lösung dann neutralisiert, so bildete sich innerhalb 18 Stunden ein Gel. Mit Wasser verdünnt bildete es kleine, im durchscheinenden Licht gut wahrnehmbare Kügelchen.

Versuchslösung b) erstarrte schon vor der Neutralisation zu einem Gel, das nur durch Zusatz von Wasser titriert werden konnte. Die Gelkügelchen adsorbierten deutlich den alkalischen Indikator Phenolphthalein.

Wurde die ursprüngliche Pektinlösung mit stärkerem, 2-*n* Alkali versetzt, so nahm die Viscosität zuerst schnell ab und wurde nach etwa 1 Stunde wieder stark viscos, um weiterhin wieder flüssiger zu werden.

In rein wässriger Lösung, sowie in Citrat-Phosphat-Pufferlösung, pH = 7, ist Pektin relativ stabil, d. h. die Viscosität einer 0,3 %igen Lösung unseres hochgereinigten Pektins nimmt innerhalb 30 Minuten (30°) um weniger als 5 % ab. Diese Abnahme wird noch geringer, wenn der Pufferlösung KCN (250 γ ml) zugesetzt wird, vermutlich weil katalysierendes Metallion gebunden (inaktiviert) wird.

Wir haben uns davon überzeugt (Tab. 1), dass die Viscositätsänderung in unseren pektinhaltigen Reaktionslösungen gegen kleinere pH-Differenzen wenig empfindlich

Tab. 1. Pektin-Konz: 4 mg/ml Reaktionslös. — 0,1 mol. Citrat-Phosphat-Puffer.

Reaktionszeit Min.	Durchflusszeiten in Minuten			
	pH 6,0	pH 7,0	pH 7,6	pH 8,0
0	9.54.8	—	—	—
0,1	—	7.47.5	8.37.0	8.35.0
10	7.53.5	7.28.5	8.15.0	8.22.6
20	7.35.2	7.23.0	7.57.2	8.09.0

¹ Dargestellt aus Schalen unreifer Zitronen. Siehe Beschreibung dieses Arkiv 8, nr 37, 376 (1955).

ist, was auch gut mit Beobachtungen von H. DEUEL übereinstimmt (Helv. Chim. Acta 26, 2004; 1943).

Einfluss verschiedener Oxydationsmittel auf die Viscositätsabnahme von Pektinlösungen

Durch die in den Tabellen 1-3 angegebenen Versuche sollen die Viscositätserniedrigungen verglichen werden, welche eintreten durch äquivalente Konzentrationen der Oxydationsmittel: Hydroperoxyd, Kaliumperjodat¹, Kaliumjodat.

Tab. 2. Pektin-Konz.: 3,1 mg/ml Reaktionslösung.

Reaktionszeit Min.	Durchflusszeit in Minuten Temp. 30°						
	Pektin Puffer	Pektin Puffer KJO ₄	Pektin Puffer KJO ₃	Pektin Puffer Ascorbin- säure	Pektin Puffer Ascorbins. KJO ₃	Pektin Puffer Ascorbins. H ₂ O ₂	Pektin Puffer H ₂ O ₂
	1	2	3	4	5	6	7
0	9,56	9,56	9,56	9,56	9,56	9,56	9,56
~0,1	9,5	1,37	—	—	—	3,23	—
5	—	0,57	—	—	—	1,23	—
10	9,38	0,47	8,54	5,48	8,23	0,51	8,38
15	—	—	—	—	—	0,48	—
20	8,55	—	8,00	4,09	7,37	—	—
30	8,27	—	7,67	3,27	6,52	—	7,78

Kaliumperjodat ist auch ohne Zusatz von Ascorbinsäure so wirksam (Spalte 2), dass es selbst bei sehr schnellen Arbeiten schwer ist, den Anfangswert der Viscosität experimentell zu bestimmen. Die Viscosität der Reaktionslösung sinkt schon in weniger als 1 Min. fast auf den Endwert.

Mit keinem anderen der geprüften Oxydationsmittel (Kaliumjodat (Spalte 3), Hydroperoxyd (Spalte 7), Natriumperborat, Kaliumpersulfat) wurde eine ähnlich starke Wirkung erreicht. Über Formol-Bildung durch Perjodat siehe KARRER u. PFAEHLER, Helv. Chim. Acta 17, 766 (1934).

In saurer Lösung wird Ascorbinsäure durch Perjodat zu DHA oxydiert.

Tab. 3. Ascorbinsäure-Konzentration 1,7 mg/ml Reaktionslösung. pH = 7.

Reaktionszeit Min.	Durchflusszeit in Minuten			
	Pektin Puffer	Pektin Puffer Asc. H ₂ O ₂ 0,63 mg	Pektin Puffer Asc. H ₂ O ₂ 0,063 mg	Pektin Puffer Asc. H ₂ O ₂ 0,0063 mg
0	10.60	10.60	10.60	10.60
~0,1	10.51.0	3.41.0	4.19.6	6.35.2
5	—	1.30.0	2.28.0	—
10	—	1.03.2	2.18.0	5.39.0
15	10.15.3	—	2.07.8	—
20	—	0.52.2	2.05.4	5.15.2
30	9.47.0	—	—	—

¹ Siehe hierzu auch H. DEUEL, Helv. Chim. Acta 26, 2002 (1943).

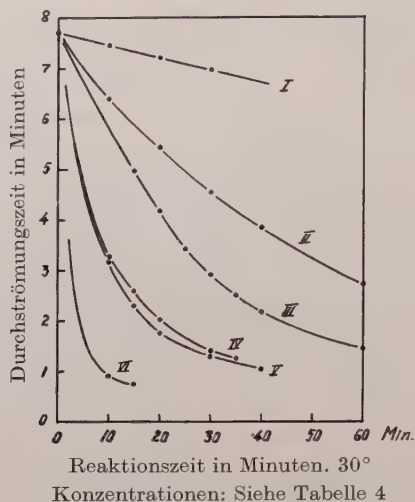


Fig. 1. Variation der Ascorbinsäure-Konz.

Lässt man KJO_4 20 Min. in Pektinlösung stehen und fällt dann das Pektin mit Aceton aus, so ist das gefällte Pektin etwas verändert.

Kaliumferricyanid sowie $\text{Fe}^{+++} + \text{H}_2\text{O}_2$ haben bei pH 7 auf Ascorbinsäure keine Wirkung.

In saurer Lösung, pH = 4, ist die Einwirkung von Hydroperoxyd auf Ascorbinsäure sehr schwach:

0,5 g Ascorbinsäure, in 5 ml Wasser gelöst, wurden mit 0,65 ml einer 30%igen Hydroperoxyd-Lösung versetzt. Nach 1 Tag war noch ein grosser Teil der Ascorbinsäure unverbraucht; die Lösung zeigte eine schwache Gelbfärbung.

Die Tatsache, dass Hydroperoxyd nur zusammen mit Ascorbinsäure imstande ist, Pektin oxydativ abzubauen, hat zu der Annahme geführt, dass diese beiden Stoffe eine Verbindung eingehen bzw. ein Ascorbinsäure-peroxyd bilden, das nach dem Massenwirkungsgesetz mit den Komponenten im Gleichgewicht steht. Diese Hypothese ist durch zwei Versuchsreihen geprüft worden, bei denen die Konzentrationen der Ascorbinsäure und des Hydroperoxyds variiert wurden, so dass sich die gleichen Konzentrationsprodukte ergaben (Fig. 2 und 3). Sofern keine Nebenreaktionen eintreten (etwa ein spontaner Verbrauch einer der beiden Reaktionskomponenten) sollte die Reaktionsgeschwindigkeit (Geschwindigkeit der Viscositätsänderung) im untersuchten pektinhaltigen System dem Konzentrationsprodukt $\text{AS} \times \text{H}_2\text{O}_2$ proportional sein.

Durch die Kombination mit Ascorbinsäure steigt der Feld-Effekt des Hydroperoxyds $\text{AS} - \text{O} \rightarrow \text{OH}$.

Aus Fig. 2 ersieht man die nahe Übereinstimmung der Kurven V und VI, in denen das Produkt $\text{AS} \times \text{H}_2\text{O}_2$ konstant = $2 \cdot 10^{-5}$ ist. Dieser Beziehung kommt vielleicht allgemeinere Gültigkeit zu. Indessen ist dieselbe schwer nachweisbar, da der Reaktionsbeginn unmessbar schnell verläuft, und da in der zweiten Hälfte der Reaktion vielleicht nicht die gleiche Molekularart des Substrates (Pektins) in Reaktion tritt wie im Anfang.

Tab. 4. Viscosität bei variierender Konzentration der L-Ascorbinsäure.

Pektinkonzentration: Lösung B.

H₂O₂: 0,63 mg/ml Reaktionslösung.

Citrat-Phosphat-Puffer. Die Acidität pH 7, kontrolliert während der Versuche, ist konstant.

Reaktionszeit	Durchströmungszeit in Minuten					
	Pektin Puffer	Pektin Puffer H ₂ O ₂	Pektin Puffer H ₂ O ₂ Asc. 1,7 mg	Pektin Puffer H ₂ O ₂ Asc. 0,17 mg	Pektin Puffer H ₂ O ₂ Asc. 0,017 mg	Pektin Puffer H ₂ O ₂ Asc. 0,0017 mg
0	7.43.0	—	—	—	—	—
~0,1	—	7.13.6	3.23.0	5.45.0	6.00.0	7.42.0
10	7.28.2	6.25.0	0.53.2	3.10.4	3.12.0	—
15	—	—	0.46.0	2.16.5	2.36.5	5.00.8
20	7.13.4	5.35.0	—	1.46.8	2.02.0	4.09.5
25	—	—	—	—	—	3.21.5
30	6.58.6	4.29.2	—	1.19.6	1.53.5	2.52.4
35	—	—	—	—	1.45.2	2.28.6
40	—	3.51.2	—	—	1.07.5	2.10.8
60	—	2.43.0	—	—	—	1.27.8
	I	II	VI	V	IV	III

Fig. 1 veranschaulicht die in dieser Tabelle angegebenen Ergebnisse.

Dehydro-ascorbinsäure (DHA)

Dieses biologische Oxydationsprodukt der Ascorbinsäure wird präparativ gewonnen durch Oxydation der Ascorbinsäure mittels Jod oder p-Chinon. Dagegen entsteht DHA nicht durch Einwirkung von Hydroperoxyd.

Im Gegensatz zu Ascorbinsäure erniedrigt DHA die Viscosität des Pektins *nicht* oder nur sehr unbedeutend.

In dem in Tabelle 5 angegebenen Versuch ist festgestellt worden, dass die DHA durch H₂O₂ wesentlich schneller als durch Perjodat angegriffen wird.

Tab. 5. Konzentrationen per ml Reaktionslösung:

Ascorbinsäure 1,7 mg; DHA 1,7 mg; KJO₄ 4,25 mg.

Pektin 2,8 mg; H₂O₂ 0,63 mg.

Citrat-Phosphat-Puffer 0,1 mol. (pH 7).

Reaktionszeit Min.	ml TR-Lösung verbraucht				
	Ascorbins. H ₂ O ₂ 1	Ascorbins. KJO ₄ 2	DHA H ₂ O ₂ 3	DHA H ₂ O ₂ Pektin 4	DHA KJO ₄ Pektin 5
0	5,3	5,3	5,3	5,3	5,3
0,1	4,5	4,2	2,2	2,2	2,2
6	3,1	0	0	0	1,7
15	2,6	—	—	—	1,6
25	2,1	—	—	—	1,3
30	1,9	—	—	—	1,2

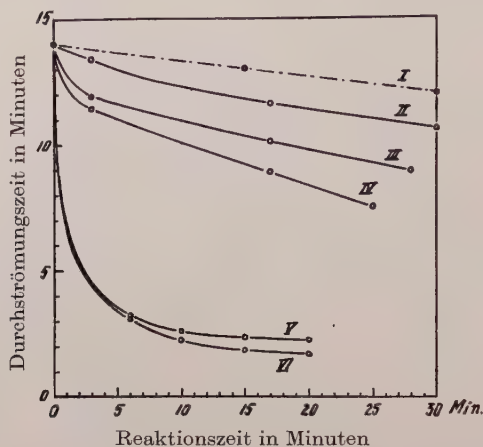


Fig. 2. Pektin-Konz.: 0,35/ml Reaktions-Lösung.

Pektin + Puffer +

Kurve I Ohne Zusatz

Kurve II AS 10^{-4}m + H_2O_2 $2 \cdot 10^{-3}\text{m}$

Kurve III AS 40^{-4}m + H_2O_2 $5 \cdot 10^{-4}\text{m}$

Kurve IV AS 10^{-3}m + H_2O_2 $2 \cdot 10^{-4}\text{m}$

Kurve V AS 10^{-2}m + H_2O_2 $2 \cdot 10^{-3}\text{m}$

Kurve VI AS 10^{-1}m + H_2O_2 $2 \cdot 10^{-4}\text{m}$

Produkt AS $\text{H}_2\text{O}_2 = 2 \cdot 10^{-7}$.

Produkt AS $\text{H}_2\text{O}_2 = 2 \cdot 10^{-5}$.

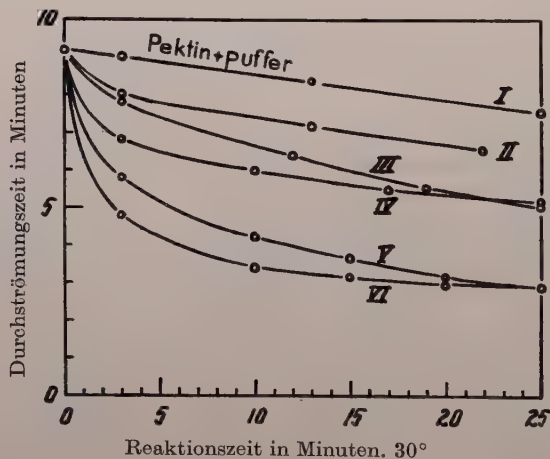


Fig. 3. Pektin: 0,35 mg/ml Reaktions-Lösung — 0,1 m. Puffer.

Pektin + Puffer +

Kurve I Ohne Zusatz

Kurve II AS 10^{-4}m + H_2O_2 $2 \cdot 10^{-2}\text{m}$

Kurve III AS 10^{-3}m + H_2O_2 $2 \cdot 10^{-3}\text{m}$

Kurve IV AS 10^{-2}m + H_2O_2 $2 \cdot 10^{-4}\text{m}$

Kurve V AS 10^{-3}m + H_2O_2 $2 \cdot 10^{-3}\text{m}$

Kurve IV AS 10^{-2}m + H_2O_2 $2 \cdot 10^{-3}\text{m}$

Produkt AS $\text{H}_2\text{O}_2 = 2 \cdot 10^{-6}$.

Produkt AS $\text{H}_2\text{O}_2 = 2 \cdot 10^{-5}$.

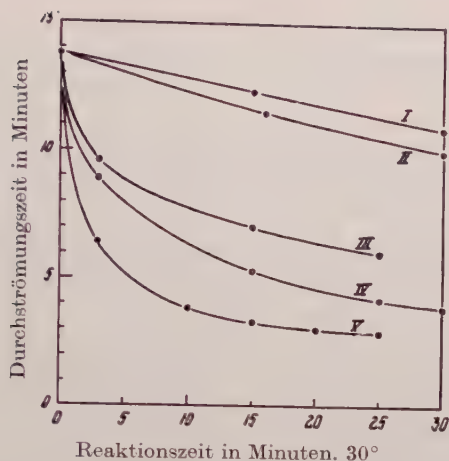


Fig. 4. Viscosität im System AS, H_2O_2 in Gegenwart von Cu^{++} .

- Kurve I 6,35 mg Pektin/ml + 0,1-m Citrat- PO_4 -Puffer.
 Kurve II Pektin + Puffer + 0,002-m H_2O_2 + 5 γ Cu^{++} /ml.
 Kurve III Pektin + Puffer + 0,001-m AS + 5 γ Cu^{++} /ml.
 Kurve IV Pektin + Puffer + 0,001-m AS + 0,002-m H_2O_2 .
 Kurve V Pektin + Puffer + 0,001-m AS + 0,002-m H_2O_2 + 5 γ Cu^{++} /ml.

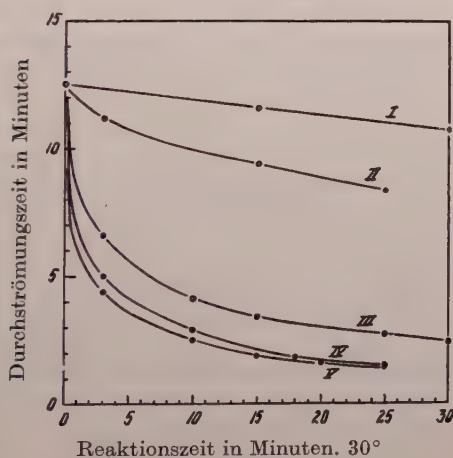


Fig. 5. Viscosität im System AS, H_2O_2 in Gegenwart von Fe^{+++} .

- Kurve I 3,90 mg Pektin/ml + 0,1-m Citrat- PO_4 -Puffer.
 Kurve II Pektin + Puffer + 0,002-m H_2O_2 + 5 γ Fe^{+++} /ml.
 Kurve III Pektin + Puffer + 0,001-m AS + 5 γ Fe^{+++} /ml.
 Kurve IV Pektin + Puffer + 0,001-m AS + 0,002-m H_2O_2 .
 Kurve V Pektin + Puffer + 0,001-m AS + 0,002-m H_2O_2 + 5 γ Fe^{+++} /ml.

Bei der Einwirkung von H_2O_2 sowie von Perjodat auf Ascorbinsäure tritt DHA als einziges Reaktionsprodukt auf.

Triose-Redukton

Dieses Redukton erniedrigt in Gegenwart von H_2O_2 die Viscosität des Pektins analog, aber in etwas geringerem Grad, wie Ascorbinsäure (siehe Arkiv Kemi 8, nr. 37, 1954).

Wie die folgenden Werte der TR-Titration zeigen, wird Triose-Redukton durch H_2O_2 oxydiert, und zwar in Gegenwart von Pektin schneller als ohne diesen Sauerstoff-Acceptor.

Reaktionszeit in Min.	TR-Lösung verbraucht, ml				
	0	6	15	25	30
Redukton H_2O_2 . . .	2,9	2,5	2,2	1,9	1,8
Redukton H_2O_2 Pektin	2,9	2,3	1,9	1,4	1,2

Oxydation von Triose-Redukton durch H_2O_2 in saurer Lösung

0,25 g Triose-Redukton in 5 ml Wasser gelöst wurden mit 0,65 ml 30 %iger Hydroperoxyd-Lösung versetzt. Beim Stehen der Reaktionslösung stieg die Temperatur auf 29° und verlief in etwa 60 Minuten. Nach 4 Stunden war das gesamte Redukton verbraucht. Oxalsäure konnte nicht nachgewiesen werden. Ein Teil der Lösung wurde mit Phenylhydrazin versetzt, worauf sofort ein rotbraunes Hydrazon ausfiel, das sich identisch mit Mesoxaldialdehyd-dihydrazon erwies.

Auch nach der Oxydation des Triose-Reduktons mit Hydroperoxyd und einer *Spur Jod*, wobei die Reaktionstemperatur auf 79° stieg, wurde das gleiche Hydrazon erhalten, dagegen keine Oxalsäure. Das gleiche Produkt erhielten wir bei der Einwirkung von KJO_4 auf Triose-Redukton in saurer Lösung.

Zur Kinetik des oxydativen Pektin-Abbaues durch L-Ascorbinsäure + Hydroperoxyd

Der experimentelle Befund, dass die Viscosität einer wässrigen Pektin-Lösung durch L-Ascorbinsäure + Hydroperoxyd schnell erniedrigt wird, besagt, dass das Hydroperoxyd aktiviert und sein Oxydationspotential erhöht wird.

Zum Verständnis dieses Effektes tragen folgende Befunde bei:

I. Bei $\text{pH} = 7$ können Ascorbinsäure + Hydroperoxyd durch zwei verschiedene Oxydationsvorgängen zweierlei Oxydationsprodukte liefern: a) Dehydro-Ascorbinsäure (DHA), b) durch Vermittlung einer Ascorbinsäure-Hydroperoxyd-Verbindung Oxalsäure.

II. Bei $\text{pH} = 4$ erfolgt dagegen die Oxydation der Ascorbinsäure (AS) stets zu DHA und zwar durch TR, Jod, Chinon, Perjodat und Hydroperoxyd + Spuren von Jod.

Ascorbinsäure + KJO_4 in saurer Lösung. 0,5 g AS in 5 ml Wasser gelöst wird mit KJO_4 in kleinen Portionen versetzt bis zur bleibenden Jodfärbung; dabei wurden angenähert 0,15 g KJO_4

verbraucht. Die so erhaltene Lösung enthielt keine nachweisbaren Mengen von Oxalsäure. Wurde die Lösung im Stickstoffstrom alkalisch gemacht, so wurde zugesetztes TR weitgehend verbraucht, was die Bildung grosser Mengen von DHA anzeigt.

Ascorbinsäure + Hydroperoxyd in saurer Lösung in Gegenwart von Spuren J_2 . 0,5 g AS in 5 ml Wasser gelöst wurden mit 0,65 ml 30 %iger Hydroperoxyd-Lösung versetzt und ein Körnchen festes Jod zugesetzt. Nach etwa 60 Minuten war die gesamte AS verbraucht. Die Lösung enthielt keine Oxalsäure sondern nur viel DHA.

III. Die Anfangsgeschwindigkeit des viscosimetrisch gemessenen Pektinabbaues steigt bei pH = 7 mit dem Produkt der Konzentrationen $AS \times H_2O_2$. Eine Proportionalität zwischen der Grösse dieses Produktes und der Geschwindigkeit der Viscositäts-Erniedrigung ist schwer nachweisbar.

IV. Die Wirkung von Ascorbinsäure – Hydroperoxyd auf Pektin ist KCN-empfindlich, woraus geschlossen werden kann, dass Cu^{++} als Katalysator an der Oxydation des Pektins beteiligt ist. Vgl. auch Fig. 4. Ebenso wirkt Fe^{+++} ; siehe Fig. 5. Über KCN-Einwirkung auf die AS-Oxydation vgl. EULER u. KLUSMANN (Arkiv 11 B nr 13 u. Z. physiol. Chem. 217, 5 (1933)).

V. *Oxydation von AS durch KJO_4 bei pH = 7.* 0,5 g AS in 5 ml Wasser gelöst und mit Alkali auf pH = 7 neutralisiert, werden portionsweise mit KJO_4 versetzt. Die Lösung zeigte dabei kleinere pH-Werte, da saure Reaktionsprodukte gebildet werden und musste deswegen während der Reaktion durch Zusätze von NaOH auf der Acidität zwischen pH 6 und 7 gehalten werden. Nun wurde KJO_4 bis zur bleibenden Jodfärbung zugesetzt. In der so erhaltenen Lösung konnten nur kleine Mengen von DHA und von Oxalsäure nachgewiesen werden.

Auch bei Versuchen, die Oxydationsprodukte nach der AS-Oxydation durch Hydroperoxyd nachzuweisen, wurden nur kleine Mengen von Oxalsäure gefunden, grössere Mengen erst nach der Vakuum-Destillation der Reaktionslösung, was darauf hindeutet, dass bei der Oxydation ein Produkt entsteht, das Oxalsäure in ziemlich lockerer Bindung enthält, die erst bei der Destillation gesprengt wird.

VI. Die Wirkung von Kaliumperjodat auf den Pektinabbau durch $AS + H_2O_2$ wird durch KCN nicht gehemmt.

VII. Die Reaktion Pyrogallol + Hydroperoxyd $\rightarrow$ Purpurogallin verläuft bei 20° an sich äusserst langsam — durch Zusatz des Enzyms Peroxydase in wenigen Minuten. Der aus Ascorbinsäure + Hydroperoxyd entstehende Komplex liefert mit Pyrogallol kein Purpurogallin, auch nicht in Gegenwart von Pektin.

Herrn Ingenieur ERIK ERIKSSON danken wir bestens für Mitarbeit bei der Ausführung der Viscositätsmessungen.

Stockholm, Universität, Institut für organ.-chemische Forschung.

Tryckt den 27 december 1955

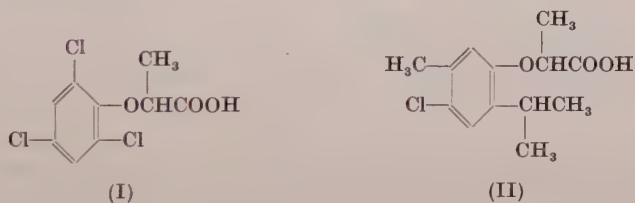
Uppsala 1955. Almqvist & Wiksells Boktryckeri AB



Optically active auxin antagonists

By MAGNUS MATELL

During the last years compounds capable of antagonizing the auxins have attracted considerable interest. Several auxins of the α -aryloxy-alkylcarboxylic acid type have been resolved into optical antipodes and their physiological effects studied (1-6). In most cases there is a considerable difference in the biological activities of the antipodes; the D-forms possess the higher auxin activity while the L-forms show no or only slight auxin effects. However, some L-forms are able to antagonize the auxins. α -Phenoxy-*n*-caproic acid behaves in a different way as both the antipodes are anti-auxins of about the same activity. For the discussion on possible mechanisms of auxin action it seemed important to study some antagonists of similar type but with pronounced biological differences between the antipodes. In this paper the resolution of α -(2,4,6-trichlorophenoxy)propionic acid (I) and α -(2-*isopropyl*-4-chloro-5-methylphenoxy)propionic acid (II) into optical antipodes with the desired antagonistic properties will be described.



The chlorothymoxy acid (II) could be resolved without complications by recrystallization of alkaloid salts. Also the (+)-form of the trichlorophenoxy acid (I) was isolated in a normal way but the separation of the (-)-form met with difficulties. Of the eleven bases tried for the resolution only (-)- β -phenylisopropylamine gave a crystalline salt containing excess of the (-)-acid. When the salt of this base with an acid containing excess of the (-)-acid was recrystallized the activity of the acid recovered from the first salt fraction decreased while the salt in the mother liquor contained a greater excess of (-)-acid. A similar result was obtained when the (+)-amine salt was recrystallized. It seemed plausible that the amine salts of the antipodes formed solid solutions or a molecular compound. To settle this question the two-component system made up of the salts of (-)- β -phenylisopropylamine with the antipodes of the acid was studied by means of the contact method (7). The salts, which melt at 162° and 170°, are not quite stable at the melting point but it was possible to establish the existence of a molecular compound made up of equal parts of (-)-amine-(+)-acid salt and (-)-amine-(-)-acid salt and melting at about 179°.

The molecular compound is thus a *partial racemate*. The final resolution was performed by successive recrystallizations of the salts contained in the mother liquors.

The plant physiological properties of the new compounds are studied by Dr. B. Åberg and the results will be published elsewhere. Dr. Åberg has kindly communicated some preliminary results. Both the acids (I) and (II) have the desired properties. Qualitatively all four antipodes resemble the homologous acetic acids but two of them, the $(-)$ -forms, show a stronger and purer anti-auxin character than the $(+)$ -forms. It has thus been demonstrated that not only auxin activity but also anti-auxin activity is dependent on sterical factors.

This result actualizes the question of the configuration of the acids described here. Some experiments on quasi-racemate formation have been carried out using the contact method. $(+)$ - α -(2,4,6-trichlorophenoxy)-propionic acid does not form a quasi-racemate with either antipode of the following acids: phenoxy-, 4-chlorophenoxy-, 2,4-dichlorophenoxy-, 2-methyl-4-chlorophenoxy-, and 2,4,5-trichlorophenoxy- α -propionic acid. The melting point of the racemic form of (I) is 115.5° while the antipodes melt at 126° , indicating a poor racemate formation tendency, which may be the main cause for the non-appearance of quasi-racemates. However, the phenoxy- and 2,4,5-trichlorophenoxy acids both have a racemate formation tendency enough pronounced to compensate the small tendency of the acid (I), which is also true to some extent regarding the 4-chlorophenoxy acid.

By a study of molecular models the author arrived at another conceivable interpretation. According to all experience the acids mentioned above resemble the 2,4,6-trichloro compound as regards structure and size enough to permit the formation of quasi-racemates. As to the spatial form of the molecule the acid (I) cannot assume a flat form as the chlorine atoms in the ortho positions restrict free rotation of the side chain, forcing it out of the plane of the ring. All the other acids have at least one free ortho position and the rotation of the side chain is not restricted which makes a flat form possible. If this flat form exists in the racemate lattice, the non-flat 2,4,6-trichloro acid cannot replace one of the antipodes without disturbing the lattice to such an extent that it becomes unstable. In the lattice of the racemic form of (I) there is evidently good possibilities to replace one of the antipodes with a structurally similar compound with an unhindered side chain. In spite of this no quasi-racemate is formed as the stability of the racemate lattice is so small, as shown by the poor racemate formation tendency, that even the slightest structural difference between the components makes the lattice unstable.

Table 1. $[\alpha]_D^{25}$ for $(+)$ -forms of α -(2-isopropyl-4-chloro-5-methylphenoxy)propionic acid (II) and α -(2,4-dichlorophenoxy)propionic acid (2,4Cl₂POP).

Solvent	$[\alpha]_D^{25}$	
	II	2,4Cl ₂ POP
Acetone	+ 30.0°	+ 35.2°
Ethanol	+ 23.3°	+ 28.1°
Chloroform	+ 20.2°	+ 21.0°
Water, Na-salt	+ 6.1°	- 14.1°
Benzene	- 1.0°	- 17.1°

It was also tried to apply the quasi-racemate method to the chlorothymoxy acid (II) but without success. Here the tendency to racemate formation is strong indeed, and the side chain is not sterically hindered but its structure differs too much from the acids available for steric comparison. However, the optical rotation in different solvents could be arranged to give a series decreasing in the same way as for α -(2,4-dichlorophenoxy)propionic acid as can be seen from Table 1. As the (+)-form of the latter has D-configuration (I), the (-)-form of (II) probably belongs to the D-series too.

A similar relation could not be found for the acid (I). It should be possible to establish its configuration by chlorination of α -(2,4-dichlorophenoxy)propionic acid, which will be reported later.

Experimental

rac.- α -(2,4,6-Trichlorophenoxy)propionic acid (I).—The synthesis of this acid has already been described in the literature (8). The acid was recrystallized from 85 % formic acid and petroleum (b.p. 85–110°). M.p. 113.5–115.5°.

94.11 mg acid; 6.70₅ ml 0.0522₄N NaOH

$C_9H_7O_3Cl_3$ calc. equiv. wt. 269.5

found „ „ 268.7

(-)- α -(2,4,6-Trichlorophenoxy)propionic acid.—To 40.4 g (0.15 mole) racemic acid dissolved in hot ethanol (750 ml) were added 50.2 g (0.15 mole) strychnine. After cooling to room temperature the solution was seeded with crystals from a fore run and was allowed to stand over night in a refrigerator. The crystalline salt was collected, the acid was isolated from a small sample and the rotatory power determined in abs. alcohol. The salt was recrystallized according to Table 2.

Table 2. Recrystallization of the strychnine salt of α -(2,4,6-trichlorophenoxy)-propionic acid.

Cryst. no.	Solvent (ml)		Yield of salt %	[α] _D of the acid
	Water	Ethanol		
1	1200	750	69	+ 6.8°
2	800	400	55	+ 11.1°
3	600	300	45	+ 16.3°
4	500	250	40	+ 15.9°
5	450	225	33	+ 17.6°
6	400	200	31.5	+ 17.8°
7	400	200	28	—
8	400	200	25	+ 17.6°

The crude (+)-acid (6.3 g) obtained from the last fraction was recrystallized from petroleum yielding 5.7 g pure (+)-acid, m.p. 125–126°.

94.01 mg acid; 6.62 ml 0.0522₄N NaOHC₉H₇O₃Cl₃ calc. equiv. wt. 269.5

found „ „ 271.8

0.2371 g dissolved in abs. *ethanol* to 20.06 ml: $2\alpha_D^{25} = +0.428^\circ$. $[\alpha]_D^{25} = +18.1^\circ$; $[M]_D^{25} = +48.8^\circ$.0.2168 g dissolved in *acetone* to 20.06 ml: $2\alpha_D^{25} = +0.649^\circ$. $[\alpha]_D^{25} = +30.0^\circ$; $[M]_D^{25} = +80.9^\circ$.0.1932 g dissolved in *benzene* to 20.06 ml: $2\alpha_D^{25} = +0.264^\circ$. $[\alpha]_D^{25} = +13.7^\circ$; $[M]_D^{25} = +36.9^\circ$.0.2069 g dissolved in *chloroform* to 20.06 ml: $2\alpha_D^{25} = +0.093^\circ$. $[\alpha]_D^{25} = +4.5^\circ$; $[M]_D^{25} = +12.1^\circ$.0.2131 g neutralized with *aqueous NaOH* and made up to 20.06 ml: $2\alpha_D^{25} = +0.016^\circ$. $[\alpha]_D^{25} = +0.8^\circ$; $[M]_D^{25} = +2.0^\circ$.

(-)- α -(2,4,6-Trichlorophenoxy)propionic acid.—The acid was regenerated from the mother liquors from the first three recrystallizations of the strychnine salt. This acid (21 g, $[\alpha]_D = -10^\circ$) and an equivalent quantity of (-)- β -phenylisopropylamine (10.6 g) were dissolved in 500 ml hot acetone. Water (40 ml) was added and the solution allowed to stand over night in a refrigerator. The salt was collected and recrystallized several times from successively smaller volumes of the same solvent. The mother liquors contained the more active products, which were again recrystallized from dilute acetone. By repeating the procedure a salt was finally obtained, which on decomposition yielded optically pure (-)-acid. After recrystallization from petroleum it melted at 125–126°.

93.45 mg acid; 6.63₅ ml 0.0522₄N NaOHC₉H₇O₃Cl calc. equiv. wt. 269.5

found „ „ 269.6

0.1925 g dissolved in abs. *ethanol* to 20.06 ml: $2\alpha_D^{25} = -0.340^\circ$. $[\alpha]_D^{25} = -17.7^\circ$; $[M]_D^{25} = -47.7^\circ$.

rac.- α -(2-isoPropyl-4-chloro-5-methylphenoxy)propionic acid (II).—Sodium (3.45 g, 0.15 g-atom) was dissolved in 120 ml abs. alcohol. 4-Chlorothymol (27.6 g, 0.15 mole) and ethyl α -bromopropionate (27.3 g, 0.15 mole) were added and the solution refluxed for two hours. 2N NaOH (200 ml) was added and the solution refluxed for another hour. The ethanol was distilled off and the residue acidified with dilute hydrochloric acid when an oil separated, which soon crystallized. The crude acid was recrystallized from 85 % formic acid yielding 18 g of the pure acid, m.p. 102–104.5°.

(-)- α -(2-isoPropyl-4-chloro-5-methylphenoxy)propionic acid.—Equimolecular quantities of racemic acid (17.7 g, 0.69 mole) and cinchonidine (20.3 g) were dissolved in a hot mixture of 700 ml acetone and 850 ml water. After some hours the salt was collected and the activity of the acid determined in the usual way. The salt was recrystallized according to Table 3.

Table 3. Recrystallization of the cinchonidine salt of α -(2-isopropyl-4-chloro-5-methylphenoxy)propionic acid.

Cryst. no.	Solvent (ml)		Yield of salt (%)	$[\alpha]_D$ of the acid
	Acetone	Water		
1	700	850	47	-13.8°
2	300	100	32	-23.6°
3	200	50	29	-23.6°
4	150	25	23	-23.2°

From the salt fraction 4, 4.4 g crude (—)-acid were isolated. It was recrystallized from 85 % formic acid. The pure acid (3.5 g) melted at 83–84.7°.

89.64 mg acid: 6.71₅ ml 0.0522₄N NaOH

C₁₃H₁₇O₃Cl calc. equiv. wt. 256.7

found „ „ 255.5

0.2170 g dissolved in abs. *ethanol* to 20.06 ml: $2\alpha_D^{25} = -0.496^\circ$, $[\alpha]_D^{25} = -22.9^\circ$; $[M]_D^{25} = -58.9^\circ$.

(+)- α -(2-*iso*Propyl-4-chloro-5-methylphenoxy)propionic acid.—8.9 g acid with $[\alpha]_D = +17^\circ$ and an equivalent quantity of morphine (10.6 g) were dissolved in a hot mixture of 75 ml *ethanol* and 200 ml *water*. After cooling crystallization was initiated by seeding with crystals from a preliminary experiment and the salt was allowed to crystallize over night. The purification of the salt was performed as seen from Table 4.

Table 4. Recrystallization of the morphine salt of α -(2-*iso*propyl-4-chloro-5-methylphenoxy)propionic acid.

Cryst. no.	Solvent (ml)		Yield of salt %	$[\alpha]_D$ of the said
	Ethanol	Water		
1.	75	200	88	+ 20.5°
2	50	200	79	+ 22.4°
3	30	100	75	+ 21.3°
4	40	125	72	+ 21.4°
5	50	130	66	+ 22.7°

The crude acid obtained from the last salt fraction was recrystallized from 85 % formic acid yielding 4.5 g pure (+)-acid, m.p. 83–84.5°.

88.34 mg acid: 6.59₅ ml 0.0522₄N NaOH

C₁₃H₁₇O₃Cl calc. equiv. wt. 256.7

found „ „ 256.4

0.2102 g dissolved in abs. *ethanol* to 20.06 ml: $2\alpha_D^{25} = +0.489^\circ$, $[\alpha]_D^{25} = +23.3^\circ$; $[M]_D^{25} = +59.9^\circ$.

0.2083 g dissolved in *acetone* to 20.06 ml: $2\alpha_D^{25} = +0.622^\circ$, $[\alpha]_D^{25} = +30.0^\circ$; $[M]_D^{25} = +76.9^\circ$.

0.2188 g dissolved in *chloroform* to 20.06 ml: $2\alpha_D^{25} = +0.440^\circ$, $[\alpha]_D^{25} = +20.2^\circ$; $[M]_D^{25} = +51.8^\circ$.

0.2146 g dissolved in *benzene* to 20.06 ml: $2\alpha_D^{25} = -0.021^\circ$, $[\alpha]_D^{25} = -1.0^\circ$; $[M]_D^{25} = -2.5^\circ$.

0.2184 g neutralized with aqueous NaOH and made up to 20.06 ml: $2\alpha_D^{25} = +0.133^\circ$, $[\alpha]_D^{25} = +6.1^\circ$; $[M]_D^{25} = +15.7^\circ$.

Melting points. Thermal analysis.—All melting points were determined with a hot stage microscope. The same apparatus was used for the study of contact preparations.

Summary

α -(2,4,6-Trichlorophenoxy)propionic acid (I) and α -(2-*iso*propyl-4-chloro-5-methylphenoxy)propionic acid (II) have been resolved into optical antipodes. The antipodes are anti-auxins and the (—)-forms show stronger physiological effects than the

(+)-forms. The salt of optically active β -phenylisopropylamine and the (+)-form of the acid (I) forms a partial racemate with the salt of the same base and the (-)-acid (I). The rotatory power of the new acids have been determined in different solvents and from these measurements it is concluded that the (+)-form of (II) belongs to the D-series. Two-component systems of the optically active forms of (I) and (II) with several structurally similar acids have been studied and the non-appearance of quasi-racemates is interpreted as due to steric hindrance of the side chain.

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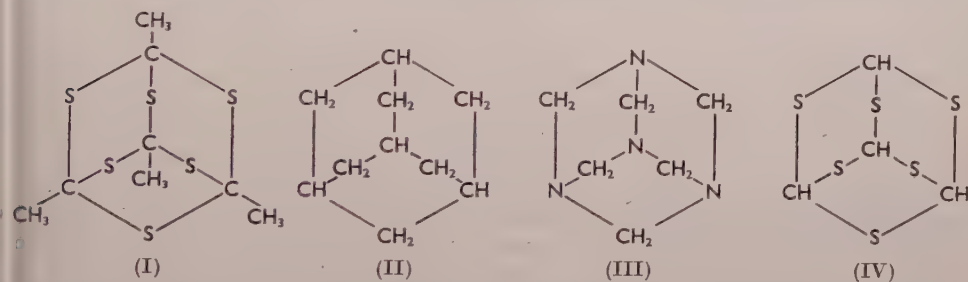
Hexathia-adamantane

By ARNE FREDGA and KJELL OLSSON

With 1 figure in the text

Some years ago, one of the present authors proposed a new formula for the so-called tetraethenyl-hexasulphide (1). According to this formula, the compound contains a strainless system of six-membered rings (I) analogous to the ring systems of adamantane (II) and hexamethylene tetramine (III). Using the nomenclature recently introduced by STETTER (2, 3), the systematic name of the compound (I) should be 1.3.5.7-tetramethyl-2.4.6.8.9.10-hexathia-adamantane. In a more recent publication, several higher homologues (up to the tetra-*n*-octyl derivative) were described (4).

It is, however, very difficult to produce conclusive evidence for the new formula. The molecule is remarkably stable, but if it is attacked by chemical agents, extensive degradation takes place. A step-wise degradation, giving well-defined products, seems impossible, and it is therefore necessary to resort to physico-chemical methods. For this purpose, it would be most convenient to investigate the unsubstituted ring system, the 2.4.6.8.9.10-hexathia-adamantane (IV), which has not been described previously.



Attempts to prepare this compound from dithioformic acid, a trimeric compound (5), or from chloroform and alkali sulphide were not successful; these experiments will not be described further. We then tried the most simple method: condensation of formic acid with hydrogen sulphide, which could be expected to take place according to the formula:



This reaction is, to a certain degree, analogous to the well-known formation of hexamethylene tetramine from ammonia and formaldehyde:

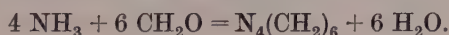
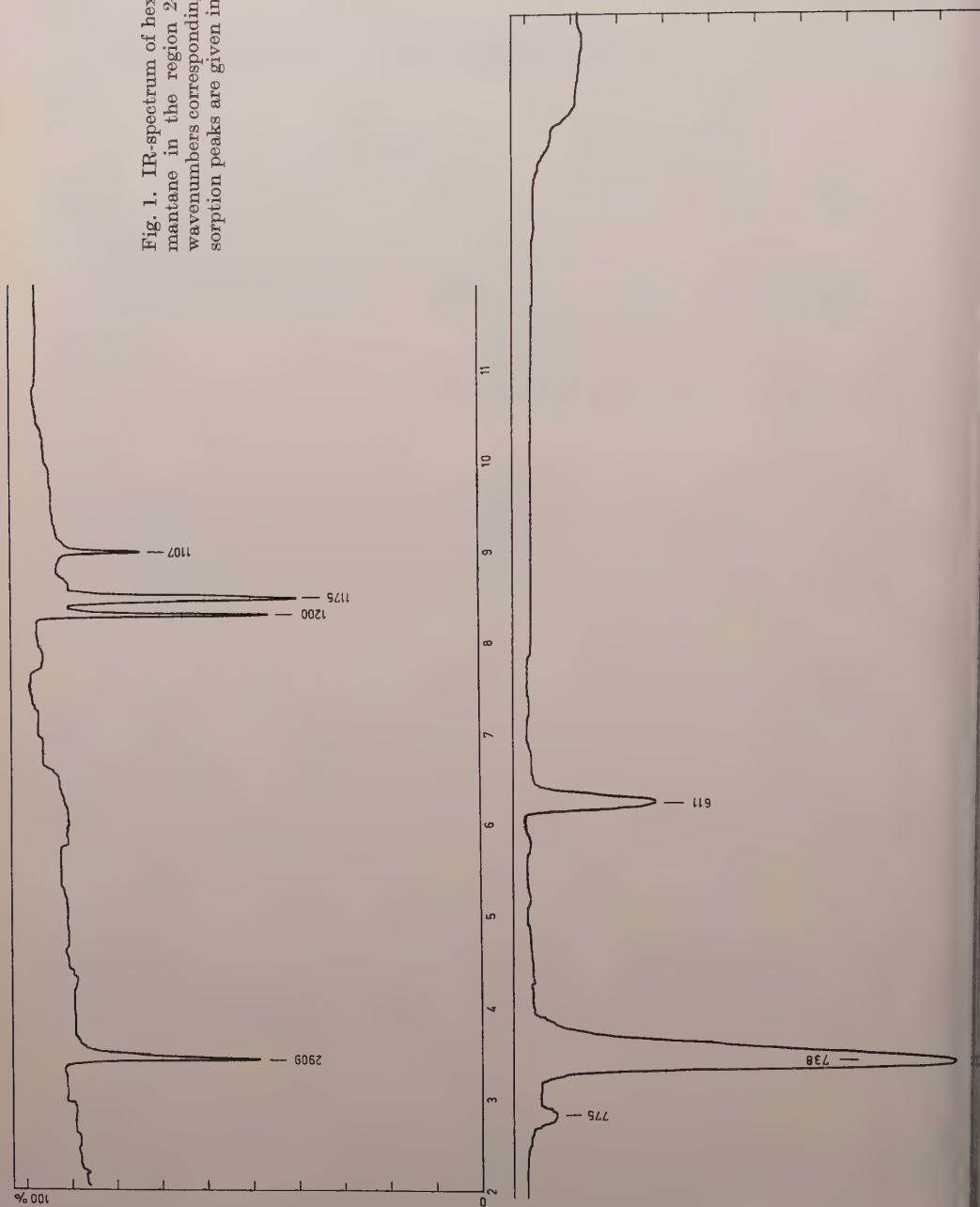


Fig. 1. IR-spectrum of hexathia-adamantane in the region 2-25 μ . The wavenumbers corresponding to the absorption peaks are given in the figure.



In experiments with formic acid and liquid hydrogen sulphide in sealed tubes, solid condensation products were easily obtained, but they were not homogeneous and in all probability they were macromolecular. In order to achieve the desired ring closure, we have therefore made use of the "dilution principle". If a solution of formic acid in nitrobenzene is saturated with hydrogen chloride and left to stand in an atmosphere of hydrogen sulphide for several weeks, a crystalline precipitate is gradually formed. The same product (though less pure) can be obtained with ether as solvent and boron trifluoride as condensing agent. The crystals are insoluble in all common solvents, but it is possible to recrystallise them from dimethyl formamide using a Soxhlet apparatus. The analytical data of the purified product are in accordance with the formula $C_4H_4S_6$. On heating, it decomposes at ca. 330° .

Owing to the extremely low solubility and volatility, the molecular weight could not be determined by current methods. Conclusive evidence for the formula could, however, be obtained by x-ray methods. The crystal structure was investigated by Dr. I. LINDQVIST and Mr. E. KROGH-ANDERSEN, who will present their results in detail in a separate publication. With their permission, the following data are given here.

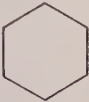
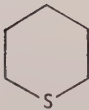
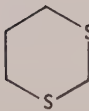
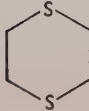
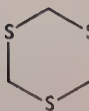
The crystals belong to the tetragonal system and the space group $P\bar{4}2_1c$. $a = b = 7.12 \text{ \AA}$; $c = 7.82 \text{ \AA}$. The unit cell contains two molecules. The six sulphur atoms form a regular octahedron as required by formula IV. The sulphur-sulphur distance *within* the molecule is $3.03 \text{ \AA}$, which could be expected for the group S-C-S with normal valency angle at the carbon atom. The *intermolecular* sulphur-sulphur distance is remarkably small, $3.5 \text{ \AA}$, indicating some kind of interaction between the sulphur atoms of adjacent molecules.

Considering the very symmetrical shape of the molecule, one could perhaps expect a higher symmetry for the crystals. Adamantane and hexamethylene tetramine are both cubic (6, 7). These molecules have, however, a more spherical shape than that of the hexathia-adamantane; this is due to the greater space requirement of the methylene groups as compared with the sulphur atoms. The crystal structure of the sulphur compound shows a certain similarity to the lattice types of adamantane and hexamethylene tetramine, but the orientation of the individual molecules is not parallel to the a and b axes. This structure permits a very close packing; the intermolecular forces exerted by the sulphur atoms are probably also of importance for the orientation of the molecules. The density is 2.07, nearly the same as that of rhombic sulphur.

The infra-red spectrum of the solid substance was studied in the region $2-25\mu$. It is very simple with only few and well-defined frequencies (Fig. 1) indicating a very symmetrical molecule. It has not been analysed in detail as yet, but the results so far are in good agreement with the formula.

It is often emphasised that adamantane and similar compounds are unusually volatile. They are also as a rule soluble in appropriate solvents. The properties of the hexathia-adamantane are therefore perhaps a little unexpected, and might lead to the suspicion that it is a high polymer. Both the crystallographic and the infra-red investigation seem, however, to exclude this possibility.

Adamantane melts at 269° and monothia-adamantane at 320° (3). Thus the introduction of one sulphur atom is sufficient to raise the melting point 50° . Organic compounds containing several sulphur atoms have often remarkably high melting and boiling points, indicating strong intermolecular forces. A comparison of some compounds with simple six-membered rings illustrates the gradual change in properties when methylene groups are replaced by sulphur atoms:

		m.p.	b.p.
	Cyclohexane C_6H_{12}	6.5°	81.5°
	Thiane (8, 9) $C_5H_{10}S$	13°	$141-142^\circ$
	1,3-Dithiane (10) $C_4H_8S_2$	53°	$207-208^\circ$
	1,4-Dithiane (11) $C_4H_8S_2$	113°	200°
	1,3,5-Trithiane (12) $C_3H_6S_3$	217°	—

The 1,3,5-trithiane containing three sulphur atoms is only sparingly soluble in benzene and carbon disulphide; it is volatile at higher temperature but no boiling point is given in the literature. The crystal structure has been examined by MOERMAN and WIEBENGA (13). In hexathia-adamantane we obviously have a similar state of affairs, but owing to the general shape of the molecule and the presence of six sulphur atoms, the peculiar properties are far more pronounced.

The difference in properties between hexathia-adamantane and its homologues is also worth mentioning. The tetramethyl derivative (I) has a well-defined melting point at $224-225^\circ$ and is easily soluble in benzene and carbon disulphide. The higher homologues have still lower melting points. Owing to the space requirement of the alkyl groups, intermolecular contact between sulphur atoms is in these cases rendered difficult if not impossible. Even here a comparison with the six-membered rings is illustrative. 1,3,5-Trithiane melts at 217° and is sparingly soluble (see above). The trimethyl and hexamethyl derivatives, generally known as trithioacetaldehyde and trithioacetone, have much lower melting points and are easily soluble in benzene and various other organic solvents (14, 15).

It should be mentioned, however, that bonding forces between sulphur and hydrogen atoms may also be of importance in this connection. The crystal structure of 1,4-dithiane, which has a rather high melting point (113°), has been investigated

recently (16). Here the intermolecular sulphur-sulphur distance is well beyond the predicted van der Waals separation. In this case, there is consequently no reason for assuming any special bonding forces between the sulphur atoms. The same holds good for the corresponding selenium compound investigated some years ago (17).

In the authors' opinion, there can be no doubt as to the existence of special bonding forces between organic molecules containing sulphur atoms. The nature of these forces is, however, not quite clear.

Experimental

Several experiments were carried out in sealed tubes under varying conditions. Formic acid was placed in the tubes, dry hydrogen sulphide was condensed over the acid at low temperature and condensation agent was added. After sealing, the tubes were left at room temperature for one to fifteen weeks. The properties of the products varied with the conditions; they were generally insoluble in common solvents but in some cases partly soluble in alkali, indicating the presence of hydroxyl or thiol groups. On continuous extraction with hot dimethyl formamide, no crystallised hexathia-adamantane could be obtained.

Preparation of hexathia-adamantane

Method I.—Nitrobenzene (700 ml) was saturated with dry hydrogen chloride and 70 ml of formic acid was added. The solution was placed in a flat dish in a large desiccator (volume ca. 8 l), which was filled with dry hydrogen sulphide at atmospheric pressure. After 34 days, the pale yellow crystalline precipitate was filtered off and washed with ethanol. The yield was 2.68 g (2.4 %). The crystals were first boiled with 50 ml of dimethyl formamide for 20 minutes. The remaining product, which was colourless, was placed in the thimble of a Soxhlet extraction apparatus and extracted continuously with 200 ml of dimethyl formamide. After about one week, practically all the substance had separated from the solution in the flask as well-shaped microscopic crystals of octahedral appearance. Yield 2 g; on heating, decomposition takes place at ca. 330°.

$C_4H_4S_6$ (244.5) calc.	C 19.65	H 1.65	S 78.70
found „	19.84, 19.71	„ 1.54, 1.85	„ 78.37, 78.22

Method II.—Anhydrous ether (200 ml) was saturated with boron trifluoride and mixed with 200 ml of formic acid in a 10 l flask. The flask was filled with dry hydrogen sulphide gas, closed with a rubber stopper, and left at room temperature. The hydrogen sulphide was renewed every 10 days. After 42 days, the precipitate was filtered off and washed with ether. Yield 15.6 g. The product contained more coloured impurities than in the first experiment; it was therefore boiled twice with dimethyl formamide and then recrystallised as described above. Yield of pure substance 5.9 g.

Preliminary experiments with other solvents (benzene, methanol, chloroform) met with no success. Attempts to replace the formic acid by its ortoester or by ethyl trithioortoformate gave no crystalline products.

The infra-red spectrum

The infra-red spectrum was studied in the region 2–25 μ . A Perkin Elmer model 21 double-beam spectrophotometer equipped with sodium chloride and potassium

bromide prisms was used for the measurements. The compound was examined as solid pressed potassium bromide plates according to the method of SCHIEDT and REINWEIN (18).

Summary

2.4.6.8.9.10-Hexathia-adamantane (IV) has been prepared from formic acid and hydrogen sulphide under special conditions. The compound forms tetragonal crystals, decomposing at ca. 330°, and is practically insoluble in all common solvents. The structure has been verified by an x-ray examination, the results of which will be published elsewhere.

The properties of the compound are discussed and attention is drawn to the intermolecular forces existing between organic molecules containing several sulphur atoms.

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Uppsala 1956. Almqvist & Wiksells Boktryckeri AB

The crystal structure of hexathia-adamantane, $(\text{CH})_4\text{S}_6$

By ERIK KROGH ANDERSEN¹ and INGVAR LINDQVIST

With 3 figures in the text

It was first suggested by Fredga (1), that the tetramethyl-hexathia-adamantane molecule, $(\text{CCH}_3)_4\text{S}_6$, has a structure analogous to hexamethylene-tetramine, $\text{N}_4(\text{CH}_2)_6$. An X-ray investigation was started to support the very convincing chemical arguments but it could not be finished at that time. Later Fredga and Olsson (2) prepared hexathia-adamantane, $(\text{CH})_4\text{S}_6$. A preliminary X-ray study with powder methods, made by von Sydow, indicated that this compound crystallized in tetragonal crystals with the cell dimensions $a = 7.120 \pm 0.014 \text{ \AA}$ and $c = 7.815 \pm 0.015 \text{ \AA}$. A complete crystal structure determination of $(\text{CH})_4\text{S}_6$ has now been carried out. It confirms the structure suggestion by Fredga. A very accurate determination of the parameters has not been attempted, nor will any of the authors have time available for further work on this crystal structure.

Crystals

The single crystals were grown by Fredga and Olsson. Twin formation prevented the use of most of them. The data used were obtained by rotation of a crystal around the c -axis. Weissenberg photographs were taken with Cu-K radiation ($l = 0-4$). The density 2.07 (determined by Olsson) corresponds to two molecules $(\text{CH})_4\text{S}_6$ in the unit cell. The Laue symmetry is $4/mmm$. Reflections $h00$ are missing for h odd and reflections hkl are missing for l odd. The space-group is thus $P\bar{4}2_1c$ (no. 114).

Relative $|F|^2$ values were calculated from the estimated intensities using Lu's curves (3). No correction was made for absorption errors.

Atomic positions

The sections $P(x, y, 0)$, $P(x, y, \frac{1}{2})$, $P(x, \frac{1}{2}, z)$ and $P(x, y, 0.28)$ were calculated on the Hägg-Laurent machine (4) and the following sulfur positions were derived:

8 S_I in 8(e) with $x = 0.133$, $y = 0.283$, $z = 0.000$

4 S_{II} in 4(c) with $z = 0.280$.

These sulfur positions would give rise to the following vectors:

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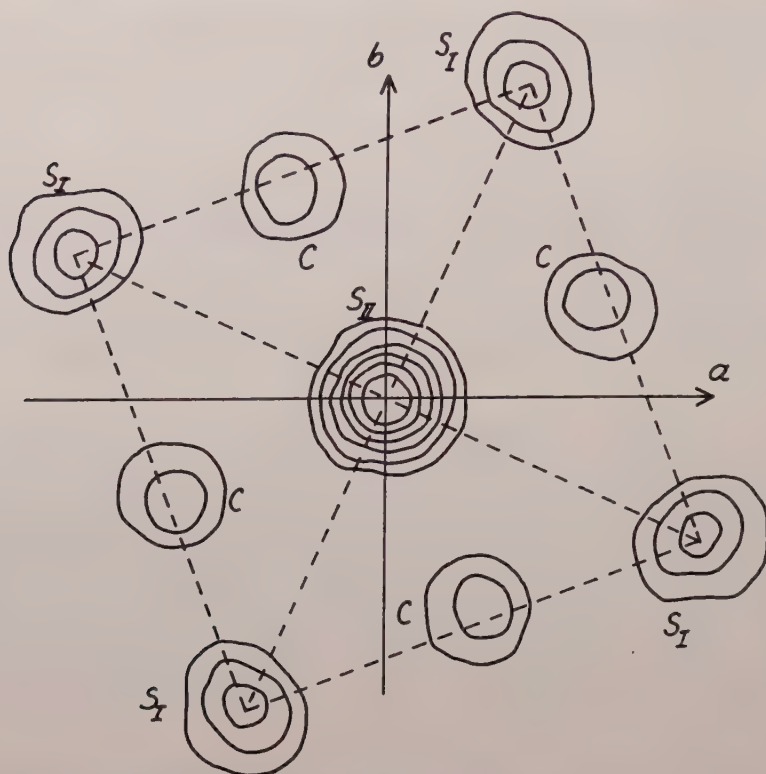


Fig. 1. Electron density projection along the c -axis, showing the projection of the S_6 octahedron and the C_4 tetrahedron which form the molecule $(\text{CH})_4\text{S}_6$.

In $P(x, y, 0)$ (In the region $x = 0-\frac{1}{2}, y = 0-\frac{1}{2}$) $\text{S}_\text{I}-\text{S}_\text{I}$ (0.266, 0.434) (0.434, 0.266) (0.416, 0.150) (0.150, 0.416)	In $P(x, \frac{1}{2}, z)$ (In the region $x = 0-\frac{1}{2}, z = 0-\frac{1}{2}$) $\text{S}_\text{I}-\text{S}_\text{I}$ (0.234, 0.500) (0.066, 0.500) $\text{S}_\text{II}-\text{S}_\text{II}$ (0.500, 0.500) (0.500, 0.060)
In $P(x, y, \frac{1}{2})$ (In the region $x = 0-\frac{1}{2}, y = 0-\frac{1}{2}$) $\text{S}_\text{I}-\text{S}_\text{I}$ (0.234, 0.500) (0.500, 0.066) (0.150, 0.150) (0.416, 0.416) $\text{S}_\text{II}-\text{S}_\text{II}$ (0.500, 0.500)	In $P(x, y, 0.28)$ (In the region $x = 0-\frac{1}{2}, y = 0-\frac{1}{2}$) $\text{S}_\text{I}-\text{S}_\text{II}$ (0.133, 0.283) (0.283, 0.133)

These vectors are all obtained at positions which are very nearly correct.

The sulfur positions were confirmed by calculation of the electron density projection $\rho(x, y)$. The carbon atom position was also found in this projection and a new calculation of $\rho(x, y)$, Fig. 1, gave the following parameters:

- 8 S_I in 8(e) with $x = 0.125, y = 0.272$
- 4 C in 8(e) with $x = 0.187, y = 0.093$

A complete structure factor calculation (using McWeeny's values (5) for the scattering factors of carbon) permitted the determination of an exponential function, $\exp[-8(\sin\theta/\lambda)^2]$, which could be used for the scaling of the observed and calculated structure factors. The value 8 in the exponent is very high. A final calculation of ρ - ρ_s gave the carbon parameters $x = 0.194$ and $y = 0.072$. This position can be checked for its agreement with the model proposed according to which the distance from C to two neighbouring S_I atoms should be equal. This gives the condition $x_C = 2.70 y_C$ while the relation found is $x_C = 2.69 y_C$. The R -value for reflections $hk0$ obtained in this way is 0.11, which is quite satisfactory.

It then remained to determine the z parameters. The function $\rho(0, 0, z)$ gave the most accurate value $z = 0.275$ for S_{II} . It was not convenient to determine the z parameter of the carbon atom from an electron density projection. The sulfur atoms do not contribute to the reflections $0kl$ with $k + l$ odd, and these are therefore very weak. The threshold value of the exposures is in fact of the same order, and a determination of the carbon atom position would require new exposures with a lower threshold intensity. The adamantane structure can, however, be checked in a very convincing way. According to this model the z_C value is determined by the condition that the shortest distances C- S_I and C- S_{II} should be equal. This gives $z_C = 0.134$. These distances should also be of the order of the calculated C-S distance 1.81 Å. In fact, they are 1.84 Å. R_{0kl} is 0.13, and the main features of the hexathia-adamantane structure can be considered as conclusively established.

Discussion of the structure

Two aspects of the crystal structure will be discussed, the structure of the molecule and the packing of the molecules.

The structure of the molecule is in agreement with Fredga's predictions (1). The S-S distances along edges of the S_6 octahedron corresponding to $S \begin{array}{c} \diagup C \diagdown \\ \diagdown S \diagup \end{array}$ links, are all 3.03 Å. In trithiane and trithioacetaldehyde Hassel and Viervoll found 3.05 Å in an electron diffraction study of the gas molecules (6). A less accurate X-ray determination of trithiane (7) gave the values 2.97 Å and 3.10 Å. Hassel and Viervoll found C-S distances of 1.81 Å, corresponding to a S-C-S angle of 114°5. This value is probably more correct than 1.84 Å found by us.

The only alternative structure suggestion for $(CH_4)_6S_6$ made by Fromm (8) contains the same octahedron of sulfur atoms. The carbon atom arrangement of that model, however, is not possible with the tetragonal symmetry $P\bar{4}2_1c$ established for this compound. This leaves no doubt about the correctness of the suggested adamantane structure.

The packing of the molecules is evident from the Figs. 2 and 3. The difference compared with adamantane and hexamethylene-tetramine has been discussed in the paper by Fredga (2). The sulfur-sulfur packing distance along the c -axis is 3.52 Å, which is shorter than the calculated van der Waals distance, 3.7 Å. Another short distance of 3.58 Å is found between S_{II} and the nearest S_I atoms of four surrounding molecules (Fig. 2). The short intermolecular distances and the dense packing (density equal to sulfur) are certainly connected with strong van der Waals interaction. This gives a simple explanation of some puzzling differences in properties between hexathia-adamantane and tetramethyl-hexathia-adamantane. The latter, which contains

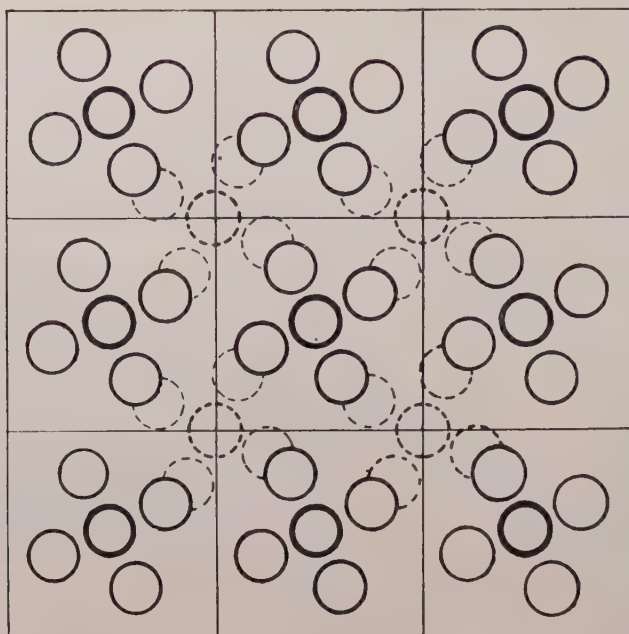


Fig. 2. The packing of the molecules perpendicular to the c -axis.

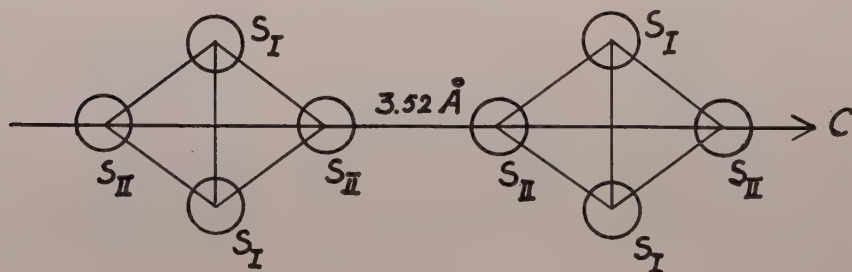
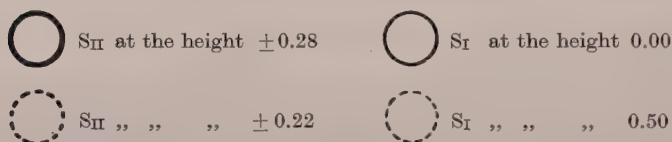


Fig. 3. The packing of the molecules along the c -axis. An edge is seen of the square formed by four S_{I} atoms.

the larger molecules, has a lower melting point and a much higher solubility in a number of solvents. The methyl groups evidently prevent the close contact between the sulfur atoms and the dense packing obtained in solid hexathia-adamantane, and in this way shield off the strong van der Waals interaction between different molecules. Moermann and Wiebenga (7) have suggested a similar explanation for the dif-

ferences in properties between trithiane and trioxane, and in the sequence trithiane, trithioacetaldehyde and trithioacetone. The intermolecular S-S distances in trithiane are 3.6 Å.

The van der Waals interaction between sulfur atoms can be expected to be stronger than between carbon atoms because of the larger polarisability of the sulfur atoms and it is tempting to try to correlate the properties of hexathia-adamantane with the rather short intermolecular S-S distances. Still shorter distances are found, however, 3.3 Å in sulfur (8) and 3.4 Å in P_4S_7 (9) which have moderate melting points and good solubilities in many solvents. The most reasonable cause of the extreme properties of $(CH)_4S_6$ then seems to be the packing which gives a favourable total van der Waals interaction, in all directions, to which particularly the sulfur atoms are contributing.

Summary

The crystal structure of hexathia-adamantane $(CH)_4S_6$ has been determined and the adamantane structure has been confirmed. The anomalous properties of the compound have been discussed in terms of the packing and of the van der Waals interaction between the molecules.

ACKNOWLEDGEMENTS

One of us (E. K. A.) wants to thank the *Royal Veterinary and Agricultural College and Nycanderska fonden* for grants which made his stay in Uppsala possible.

The authors are greatly indebted to Prof. A. FREDGA for the suggestion of the problem and for valuable discussions during the work, and to Dr. VON SYDOW for the information about the cell dimension data. Finally we want to thank Prof. G. HÄGG for the facilities put at our disposal.

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Studies on the hydrolysis of metal ions

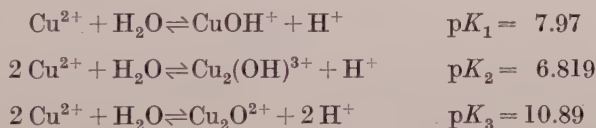
Part 13. The hydrolysis of the copper(II) ion, Cu^{2+}

By CLARA BERECKI-BIEDERMANN

With 5 figures in the text

The hydrolysis of the copper(II) ion has been studied by a number of investigators either with a glass electrode [1, 2, 2 b, 3] or a quinhydrone electrode [4, 5, 6, 7]. All these investigators—with the notable exception of Pedersen [8]—measured the “pH” of solutions of “pure” copper(II) salts (e.g. with ClO_4^- , SO_4^{2-} , NO_3^-) and summarized their results by setting up empirical relationships between the “pH” and the total Cu concentrations. However, no effort was made to apply the law of mass action.

In 1943, K. J. Pedersen [8] made an extensive investigation of this subject at 18 °C using the glass electrode in $\text{Cu}(\text{NO}_3)_2$ solutions of varying ionic strength. He realized the difficulties associated with the measurement of small acidity constants and paid particular attention to the accurate determination of small amounts of acid or base present in the stock solution of $\text{Cu}(\text{NO}_3)_2$; the neglect of this very important point made previous investigators unsuccessful in this field. Pedersen could explain the results assuming the equilibria:



The values of $\text{p}K$ given are valid at infinite dilution.

In the present work the hydrolysis equilibria have been investigated by the experimental and mathematical methods worked out in this laboratory [9, 10].

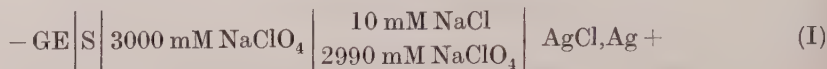
Method

All measurements were made on solutions “S” of the following general composition

$$[\text{Cu}(\text{II})_{\text{total}}] = B \text{ mM}, \quad [\text{H}^+] = H \text{ mM}, \quad [\text{Na}^+] = (3000 - 2B - H) \text{ mM}, \\ [\text{ClO}_4^-] = 3000 \text{ mM}$$

where mM denotes millimoles per liter, B is the total concentration of copper(II) and H the analytical concentration of hydrogen ions (assuming that all Cu(II) is present as Cu^{2+}). The values of B chosen were 1.00, 10.00, 25.00, 50.00, 100.0 mM, and H varied between the limits 10 and -2.2 mM.

The equilibrium concentration of $\text{H}^+ = h$ was determined by measuring the emf of the cell



which may be written



(GE = glass electrode, SE = silver half-cell).

Assuming the activity factors to be constants the emf of this cell at 25°C can be written:

$$E = E_0 - 59.16 \log h - E_j \quad (1)$$

where E_0 is a constant and E_j is the liquid junction potential. It has been shown [9] that E_j is a function of h only, as long as h and B are low compared to $[\text{ClO}_4^-]$. This condition is fulfilled in this work. $E_j = -0.0167 h$ mV was taken from [9], which proved to give a constant E_0 .

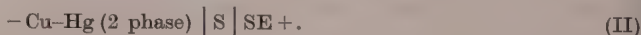
The measurements were carried out as a series of potentiometric titrations, starting with a solution S_1 of known H and successively adding NaOH ("T"). The total concentration of copper was kept constant by the simultaneous addition of an equal volume of a solution S_2 containing $2B$ Cu(II). All the solutions were made up to contain $[\text{ClO}_4^-] = 3000$ mM.

From these measurements the average number of OH bound per Cu

$$\frac{h - H}{B} = Z$$

could be calculated as a function of h .

At the beginning of this investigation attempts were made to determine the equilibrium concentration of Cu^{2+} by measuring the emf of the cell:



However, even at high values of h , where the hydrolysis is negligible, the emf values drifted and small red crystals of Cu_2O appeared on the surface of the amalgam because of the reaction $\text{Cu}^{2+} + \text{CuHg(s)} + \text{H}_2\text{O} \rightleftharpoons \text{Cu}_2\text{O(s)} + 2\text{H}^+ + \text{Hg(l)}$.

Attempts to use the quinhydrone electrode also failed; just before the hydrolysis of the Cu^{2+} became appreciable, the emf began to drift slowly, and in some cases no constant value was attained even after several days. The drift was probably due to a slow reaction between Cu(II) and quinhydrone; it was shown with a glass electrode that the reaction was accompanied by an increase in h .

Reagents and analysis

Copper(II)oxide (Schering, p.a.) was carefully tested for impurities, especially SO_4^{2-} , Fe(III) , and Cl^- ; the tests for all these ions were negative.

Copper(II)perchlorate solution was prepared by dissolving CuO in excess of hot HClO_4 (p.a. 70 %). A small amount of Cl^- could be found in this solution; probably the CuO contained some metallic Cu which reduced some of the ClO_4^- . The HCl was expelled by evaporating the solution with a large excess of HClO_4 until $\text{Cu(ClO}_4)_2$ crystals were formed in the hot solution. After cooling, water was added. The stock solution prepared in this way was filtered through a sintered glass crucible before further use.

The copper(II) content of the stock solution was measured by electrodeposition after adding H_2SO_4 . The accuracy was better than $\pm 0.1\%$. The total ClO_4^- content of the stock solution was determined by passing a definite volume through the ion exchanger Duolite C3, and titrating the eluate with standard NaOH .

The analytical excess of H^+ in the stock solution was calculated from $[\text{H}^+] = [\text{ClO}_4^-] - 2[\text{Cu(II)}]$; since this was a small difference between two large numbers one could not expect it to be more accurate than about 1.0 %. This accuracy did not prove to be satisfactory for the present purpose. In each series of measurements the initial H was determined more accurately by a graphical method described in the next section.

Solutions of NaOH , HClO_4 , NaClO_4 and NaHCO_3 were prepared and analyzed as usual in this laboratory [11]. Analyses were made with an accuracy of $\pm 0.1\%$.

Sodium chloride (Merck, p.a.) was used after drying at 250°C ; the Br^- content was less than 0.01 %.

Copper amalgam 2 % (two phases: CuHg(s) and practically pure Hg(l)) was made by electrolysis.

The *quinhydrone* preparation (Merck, p.a.) contained some Fe(III) which could be removed by recrystallization from hot water.

Nitrogen was taken from a cylinder. It was freed from O_2 by hot activated Cu [12] and from CO_2 with "Ascarite", and was finally bubbled through 3M NaClO_4 solution to obtain the correct water vapor pressure.

The determination of the analytical concentration of hydrogen ions

The results found in this work are based on the analysis of the function $Z(\log h)_B$ which was determined experimentally. In order to obtain reliable values of Z the analytical concentration of hydrogen ions, H , must be known very accurately. For this purpose a special potentiometric titration method was developed using the cell:



When hydrolysis in S can be neglected the emf of this cell will be given by

$$E = E_{0Q} + 59.16 \log H + E_{1Q} \quad (2)$$

where E_{0Q} is constant, and E_{jQ} is the liquid junction potential plus a small correction for the basicity of quinone [9].

As an example let us suppose that accurate values of the analytical concentration of hydrogen ions in solutions S_1 and S_2 (denoted by H_1 and H_2) are required, and that approximate values of these quantities (H'_1 and H'_2) are known, e.g. from analysis with the ion exchanger.

The titration consists of two parts. In part 1, the analytical hydrogen ion concentration of S_1 is decreased in several steps by adding equal volumes of a carefully standardized NaOH solution, (T_1), and of S_2 . The lower limit of the concentration of hydrogen ions in S_1 , H'_0 , is determined by the condition that the hydrolysis should still be negligible. In the second part (part 2), one adds instead of T_1 a strong, standardized $HClO_4$ solution (T_2) and the concentration of hydrogen ions is increased stepwise so that it exceeds by far the starting value in part 1. H in part 2 is only slightly influenced by errors of H'_1 and H'_2 so that an accurate value of E_{0Q} can be obtained from equation (2). With this value of E_{0Q} an accurate value of the analytical concentration of hydrogen ions can be calculated for each point of part 1 which in turn gives H_1 and H_2 .

Data for one titration are given in full in Table 1. It is seen that in part 2, E_{0Q} is constant within ± 0.05 mV; a higher accuracy can hardly be expected with the equipment used.

Table 1. Titration for determining the analytical concentration of hydrogen ions in S_1 and S_2 .

S_1 : $B = 25.00$ mM, $H'_1 = 10$ mM, $[Na^+] = 2940$ mM, $[ClO_4^-] = 3000$ mM, $v_1 = 49.62$ ml

S_2 : $B = 50.00$ mM, $H'_2 = 10$ mM, $[Na^+] = 2890$ mM, $[ClO_4^-] = 3000$ mM, v_2 ml

T_1 : $[NaOH] = 19.85$ mM, $[NaClO_4] = 3000$ mM, v_3 ml

T_2 : $[H^+] = 600.0$ mM, $[Na^+] = 2400$ mM, $[ClO_4^-] = 3000$ mM, v_4 ml

$$E_{0Q} = 178.15 \pm 0.05 \text{ mV}$$

	v_2 ml	v_3 ml	v_4 ml	H' mM	H mM	$-E_{jQ} =$ $0.018 H$ mV	E mV	$E - 59.16 \times$ $\log H - E_{jQ}$ mV	H with $E_{0Q} =$ $= 178.15$ mV	H calculated with $H_1 = 9.76$ mM $H_2 = 9.13$ mM
Part 1	—	—		10.0		0.18	236.4 ₇	(using H') 177.4 ₉	9.74 ₈	9.76 ₀
	10.00	10.00		5.7 ₁		0.10	221.4 ₈	176.8 ₁	5.42 ₂	5.41 ₇
	15.00	15.02		4.3 ₇		0.08	214.0 ₅	176.2 ₃	4.05 ₇	4.05 ₇
	20.00	20.02		3.3 ₃		0.06	206.3 ₃	175.4 ₈	3.00 ₂	3.00 ₈
	25.00	25.05		2.5 ₀ = H'_0		0.04	197.8 ₇	174.4 ₀	2.15 ₉	2.16 ₀
Part 2	35.00		10.02		53.1 ₅	0.96	279.2 ₅	(using H) 178.1 ₃		
	40.00		15.03		72.6 ₁	1.31	286.9 ₄	178.1 ₅		
	45.00		20.04		89.2 ₈	1.61	291.9 ₅	178.1 ₅		
	50.00		25.03		103.6 ₅	1.87	295.5 ₀	178.1 ₃		
	55.00		29.96		116.0 ₅	2.09	298.1 ₅	178.1 ₀		
	60.00		34.98		127.2 ₄	2.29	300.3 ₈	178.1 ₆		
	65.00		39.98		137.1 ₄	2.47	302.1 ₂	178.1 ₆		
	71.00		46.01		147.7 ₂	2.66	303.8 ₀	178.1 ₂		

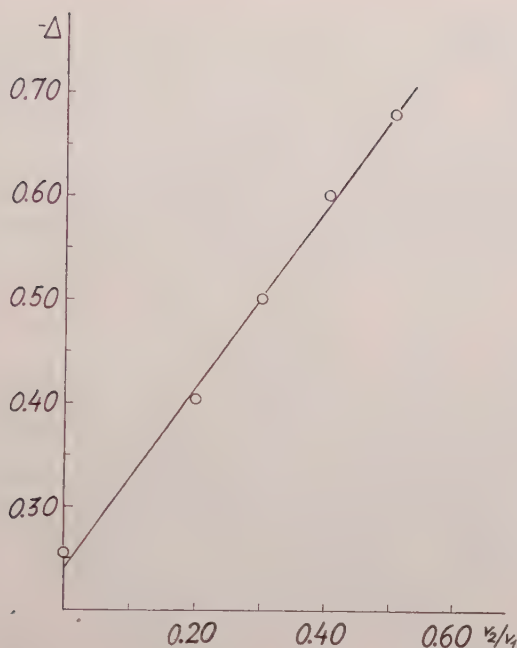


Fig. 1. Δ as a function of v_2/v_1 . The line represents the equation $\Delta = -0.24 - 0.87 (v_2/v_1)$.

H_1 and H_2 can be conveniently found by a graphical method. For each point in part 1, the concentration of hydrogen ions is given by the equation

$$H = 10^{(E - E_{0Q} - E_{1Q})/59.16} = \frac{v_1 H_1 + v_2 H_2 - v_3 c}{v_1 + v_2 + v_3}. \quad (3)$$

Here H_1 and H_2 denote the accurate H in solutions S_1 and S_2 which are to be determined, c is the $[\text{OH}^-]$ in T_1 , v_1 = ml of S_1 , v_2 = ml of S_2 and v_3 = ml of T_1 . Equ. (3) can be rearranged:

$$H_1 + \frac{v_2}{v_1} H_2 = \frac{H (v_1 + v_2 + v_3)}{v_1} + \frac{v_3}{v_1} c. \quad (4)$$

An approximate value of H , denoted here by H' , is known from the added volumes of the solutions S_1 , S_2 and T_1 and using the approximate values (H'_1 and H'_2) of the hydrogen ion concentration in S_1 and S_2 :

$$H' = \frac{v_1 H'_1 + v_2 H'_2 - v_3 c}{v_1 + v_2 + v_3}. \quad (5)$$

Introducing δ_H , δ_1 and δ_2 , the errors of H' , H'_1 and H'_2 , equ. (5) can be written

$$(H_1 - \delta_1) + \frac{v_2}{v_1} (H_2 - \delta_2) = (H - \delta_H) \frac{v_1 + v_2 + v_3}{v_1} + \frac{v_3}{v_1} c. \quad (6)$$

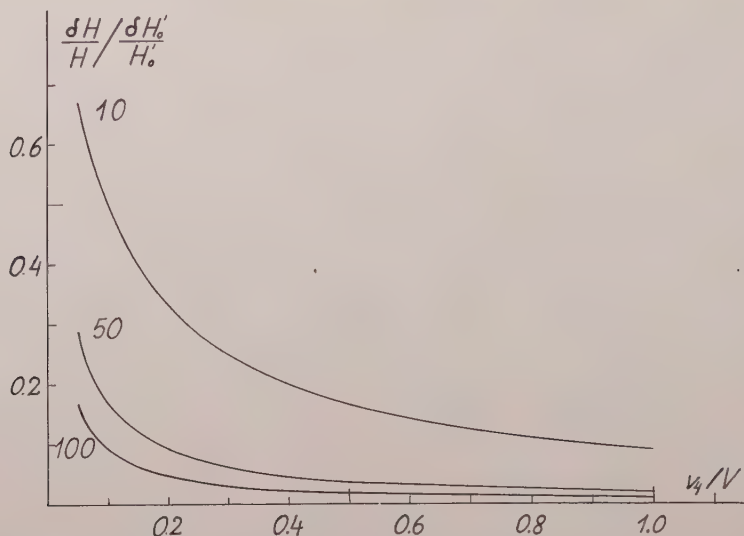


Fig. 2. $(\delta H/H)/(\delta H'_0/H'_0)$ as a function of $v_4 \times V^{-1}$. The figures on the curves give the value of the parameter $(H_4 + H'_2)/H'_0$.

Subtracting equ. (6) from (4)

$$\delta_1 + \frac{v_2}{v_1} \delta_2 = \delta_H \frac{v_1 + v_2 + v_3}{v_1} = \Delta = (10^{(E - E_{0Q} - E_{1Q})/59.16} - H') \frac{v_1 + v_2 + v_3}{v_1}.$$

If Δ is plotted as a function of v_2/v_1 the intercept of the resulting straight line gives δ_1 and the slope δ_2 . This graphical method of evaluation is shown in Fig. 1 for the titration given in Table 1.

The accuracy of H_1 and H_2 obtained in this way will depend on that of H in part 2. The analytical concentration of hydrogen ions in part 2 is calculated from the equation

$$H = \frac{V H'_0 + v_4 (H_4 + H'_2)}{V + 2 v_4}$$

where $H'_0 = \frac{v_1 H'_1 + v_2 H'_2 - v_{3 \max} c}{V}$, $V = v_1 + v_2 + v_{3 \max}$, H_4 is the $[H^+]$ of the standardized $HClO_4$ solution, (T_2), and v_4 = added volume of $HClO_4$ in ml.

Assuming that the relative errors in V , v_4 and in $(H_4 + H'_2)$ are negligible compared with $\delta H'_0/H'_0$, the relative error of H in part 2 will be

$$\frac{\delta H}{H} = \frac{\frac{\delta H'_0}{H'_0}}{1 + \frac{v_4}{V} \left(\frac{H_4 + H'_2}{H'_0} \right)}.$$

Fig. 2 shows $(\delta H/H)/(\delta H'_0/H'_0)$ the increase of relative accuracy in part 2 as a function of v_4/V for varying values of the parameter $(H_4 + H'_2)/H'_0$. In the titration described in Table 1, $(H_4 + H'_2)/H'_0$ was 244. It is seen from Fig. 2 that in a rather unfavorable case, when $(H_4 + H'_2)/H'_0$ is as small as ten, H in part 2 can easily be made ten times as accurate as H'_0 . The functions plotted may be used to find the necessary conditions for a desired accuracy.

This method may be applied generally to determine the concentration of a strong acid in the presence of a weaker one. When the quinhydrone electrode is unsuitable it may be replaced by a glass or hydrogen electrode.

Apparatus

The emf was measured (with an accuracy of about ± 0.2 mV) by means of a valve potentiometer, Radiometer PHM3, using glass electrode half-cells. The valve potentiometer was calibrated against a Leeds and Northrup potentiometer, type K. The latter, which permitted the estimation of hundredths of a mV, was used to measure the emf of cells with copper amalgam or quinhydrone.

Glass electrodes were supplied by Mr. E. Blomgren, Uppsala. These electrodes functioned very satisfactorily, attained equilibrium within 5–10 minutes, and remained constant for several hours within 0.2–0.3 mV.

Ag, AgCl electrodes were prepared according to Brown [13]. The HCl solution used for electrolysis was freed from Br^- as described in [11].

In the copper amalgam half-cells, care was taken to avoid any oxidation of the amalgam, which was introduced into the cell in an atmosphere of O_2 -free CO_2 . In quinhydrone half-cells, two bright Pt foils were employed simultaneously.

During the measurements the solutions were stirred by a stream of N_2 .

The reference electrode vessel and the salt bridge were of the type described in [14].

The cells were kept in a paraffin oil thermostat at $25.0 \pm 0.1^\circ\text{C}$, and the thermostat was placed in a thermostat room at 25°C .

Results

Each series of measurements ("titration") started from a solution S_1 of $H \approx 10$ mM and total copper concentration B , to which equal volumes of a solution S_2 containing $2B$ mM Cu(II) and of another "T" containing NaOH were added stepwise.

E_0 was calculated from the first 5–10 points where the hydrolysis is negligible ($h \geq 0.2$ mM), so one may assume that $h = H$; in general, E_0 was constant within 0.1–0.2 mV. The equilibria were established rapidly; the potential became steady after 5–10 minutes and remained constant for 1–2 hours. For a few typical points selected for further investigation, this constancy was found to hold even over a period of 24 hours. On repeating a titration, $E - E_0$ was reproducible within the accuracy of the potentiometer.

To decide whether the equilibria are real, points were taken in the course of a few series by adding HClO_4 instead of NaOH . In this way the same h

was reached from two opposite directions, first decreasing H then increasing H . In all cases the $E - E_0$ values coincided within the experimental error (0.1–0.2 mV).

The titration was continued until a visible precipitate was formed; and in some cases a few points were taken when solid phase was certainly present. These points are shown in parenthesis in Figs. 3 and 4. The precipitate present in the last point was filtered and weighed. For instance, for $B = 100.0$ mM ($Z = 0.02$) the weight of precipitate was less than 0.1–0.2 mg corresponding to less than 0.1 % of B ; in the last point with $B = 50.0$ mM it was 1 % ($Z = 0.045$), with $B = 25.0$ mM 2 % ($Z = 0.06$), with $B = 10.0$ mM 0.5 % ($Z = 0.10$). These figures indicate the maximum possible error in Z , even in the last (discarded) points. Though the solutions were very carefully examined, no solid phase could be detected except in the last 2–3 points of a series of measurements. If appreciable amounts of precipitate had been formed before the last point, the $Z(\log h)_B$ curve would have run practically parallel with the Z axis (see [10] ABII, equ. 17 a). The last point for $B = 25.0$ and 50.0 mM in Fig. 3 indicates this tendency.

Table 2. Measurements with $B = 25.00$ mM.

S₁: $B = 25.00$ mM, $H_1 = 9.76$ mM, $[\text{Na}^+] = 2940$ mM, $[\text{ClO}_4^-] = 3000$ mM, 49.62 ml

S₂: $B = 50.00$ mM, $H_2 = 9.14$ mM, $[\text{Na}^+] = 2890$ mM, $[\text{ClO}_4^-] = 3000$ mM, v_2 ml

T: $[\text{NaOH}] = 19.85$ mM, $[\text{NaClO}_4] = 3000$ mM, v_3 ml

$$E_0 = 132.2 \pm 0.3 \text{ mV}$$

v_2 ml	v_3 ml	H mM	$-E_j$ mV	$-E_G$ mV	E_0 mV	h mM	$h - H$ mM	Z
—	—	9.76	0.16	73.8	132.1	9.76 ₉	0.009	0.000 ₄
9.99	10.02	5.40 ₈	0.09	88.8	132.1	5.43 ₄	0.034	0.001 ₄
15.00	15.03	4.05 ₈	0.07	96.3	132.2	4.05 ₅	−0.001	0.000 ₀
20.00	20.05	3.00 ₁	0.05	104.4	132.6	2.95 ₆	−0.046	−0.001 ₈
25.00	25.03	2.16 ₈	0.03	112.4	132.2	2.16 ₄	−0.004	−0.000 ₂
30.00	30.06	1.47 ₅	0.03	121.9	131.9	1.49 ₅	+0.020	+0.000 ₈
35.03	35.06	0.90 ₇	0.02	134.8	132.3	0.90 ₄	−0.003	−0.000 ₁
38.00	38.06	0.605	0.01	145.1	132.2	0.605	0.000	0.000 ₀
40.00	40.06	0.422	0.01	154.2		0.425	+0.003	+0.000 ₁
41.00	41.06	0.334 ₁		159.8		0.341 ₆	0.007 ₅	0.000 ₃
42.00	42.17	0.232 ₄		167.6		0.252 ₁	0.019 ₇	0.000 ₈
43.00	43.04	0.169 ₆		175.4		0.186 ₁	0.016 ₅	0.000 ₇
44.00	44.07	0.085 ₅		188.9		0.110 ₁	0.025 ₁	0.001 ₀
45.00	45.07	0.0071 ₆		207.6		0.053 ₂	0.046 ₀	0.001 ₈
46.00	46.08	−0.0706		226.3		0.0257	0.096 ₃	0.003 ₉
47.00	47.10	−0.146 ₁		238.1		0.0162	0.162 ₃	0.006 ₅
48.00	48.08	−0.215 ₅		244.5		0.0126	0.228 ₁	0.009 ₁
49.00	49.08	−0.284 ₄		249.2		0.0105	0.295 ₀	0.011 ₈
50.00	50.07	−0.351 ₄		252.6		0.0092 ₂	0.360 ₆	0.014 ₄
51.00	51.07	−0.417 ₃		253.2		0.0090	0.426 ₃	0.017 ₀
52.00	52.07	−0.481 ₅		255.6		0.0082	0.490	0.019 ₆
62.00	62.10	−1.046		267.0		0.0052 ₉	1.051	(0.042 ₀)
72.00	72.13	−1.494		266.9		0.0053 ₁	1.499	(0.060 ₀)

Table 3. Survey of measurements.

$B = 1.000 \times 10^{-2} \text{ M}$		$B = 2.50_0 \times 10^{-2} \text{ M}$		$B = 5.00_0 \times 10^{-2} \text{ M}$		$B = 1.000 \times 10^{-1} \text{ M}$			
$-\log h$	Z	$-\log h$	Z	$-\log h$	Z	$-\log h$	Z	$-\log h$	Z
4.862 ₈	0.005 ₈	3.569 ₆	0.000 ₃	4.466 ₅	0.000 ₃	3.769 ₁	0.000 ₃	3.542 ₆	0.000 ₂
5.153 ₅	0.012 ₃	3.924 ₆	0.001 ₁	4.598 ₄	0.000 ₈	3.990 ₅	0.000 ₆	3.708 ₂	0.000 ₄
5.217 ₇	0.015 ₇	4.228 ₉	0.003 ₀	4.730 ₂	0.000 ₇	4.115 ₆	0.000 ₉	3.885 ₇	0.000 ₈
5.278 ₆	0.019 ₁	4.737 ₆	0.006 ₃	4.958 ₄	0.001 ₀	4.233 ₉	0.001 ₄	4.061 ₅	0.001 ₄
5.320 ₈	0.022 ₅	5.097 ₇	0.012 ₂	5.274 ₅	0.001 ₈	4.345 ₅	0.002 ₀	4.188 ₃	0.002 ₂
5.359 ₇	0.025 ₇	5.266 ₇	0.019 ₀	5.590 ₆	0.003 ₉	4.423 ₃	0.002 ₇	4.362 ₄	0.003 ₉
5.393 ₅	0.029 ₁	5.364 ₈	0.025 ₇	5.790 ₀	0.006 ₅	4.546 ₆	0.004 ₀	4.460 ₄	0.005 ₆
5.427 ₃	0.032 ₉	5.437 ₅	0.032 ₈	5.898 ₂	0.009 ₁	4.570 ₃	0.004 ₂	4.529 ₈	0.007 ₂
5.473 ₀	0.038 ₈	5.484 ₈	0.038 ₇	5.977 ₇	0.011 ₈	4.702 ₂	0.006 ₃	4.636 ₂	0.010 ₂
5.513 ₅	0.044 ₉	5.518 ₆	0.045 ₀	6.035 ₂	0.014 ₄	4.790 ₀	0.008 ₉	4.711 ₆	0.013 ₅
5.537 ₂	0.050 ₈	5.549 ₀	0.050 ₈	6.045 ₃	0.017 ₀	4.850 ₉	0.011 ₂		
5.555 ₈	0.056 ₇	5.572 ₇	0.056 ₇	6.085 ₉	0.019 ₆	4.920 ₂	0.014 ₄		
5.577 ₈	0.062 ₆	5.591 ₃	0.062 ₆	(6.276 ₉ 0.042 ₀)		4.989 ₅	0.019 ₃		
		5.608 ₃	0.068 ₂	(6.275 ₂ 0.060 ₀)		5.030 ₁	0.023 ₈		
		5.633 ₅	0.073 ₇			5.074 ₀	0.027 ₈		
		5.636 ₅	0.079 ₀			5.101 ₁	0.031 ₆		
		(5.660 ₈ 0.084 ₂)				(5.140 ₀ 0.038 ₀)			
		(5.714 ₇ 0.099 ₂)				(5.057 ₁ 0.043 ₆)			
								4.399 ₆	0.004 ₂
								4.431 ₇	0.004 ₇
								4.460 ₄	0.005 ₃
								4.504 ₄	0.006 ₃
								4.637 ₉	0.010 ₂
								4.712 ₃	0.013 ₆
								4.778 ₂	0.017 ₄
								(4.828 ₉ 0.021 ₉)	

The solubility equilibria were quickly established. For the last point, where a solid phase was undoubtedly present, the emf slowly decreased by a few mV during the first 1–2 hours, but then remained constant for at least 24 hours.

Complete data for one titration are shown in Table 2. In Table 3, all the experimental data are summarized by giving the sets (Z , $\log h$, B). In Fig. 3 Z is plotted as a function of $\log h$ for the B values studied.

The relative error in Z depends primarily on $|h - H|$

$$\left| \frac{\delta Z}{Z} \right| = \frac{|\delta h| + |\delta H|}{|h - H|} + \left| \frac{\delta B}{B} \right|.$$

When $|h - H|$ is small compared to H , Z is rather uncertain but as $(h/H) \rightarrow 0$ $\delta Z/Z$ tends to a minimum determined by the accuracy of analysis. It can be estimated from the accuracy of our analyses that $|\delta H/H| = 0.002$, $|\delta B/B| = 0.002$, so when h/H tends to 0, δZ will attain its minimum value 0.004 Z . The values of Z were found to be reproducible within 0.001 to 0.002 units.

With $B = 1.00 \text{ mM}$ the spread in Z was too large, because of the very low buffer capacity of such a dilute solution.

Calculation of the hydrolysis constant

To evaluate the measurements the systematic approach outlined by Sillén [10] was followed. From Fig. 3 $(\Delta \log B / \Delta \log h)_Z = 2$, within the limits of experimental error. If this is valid then Z must be a function of $[\text{Cu}^{2+}] h^{-2}$ only.

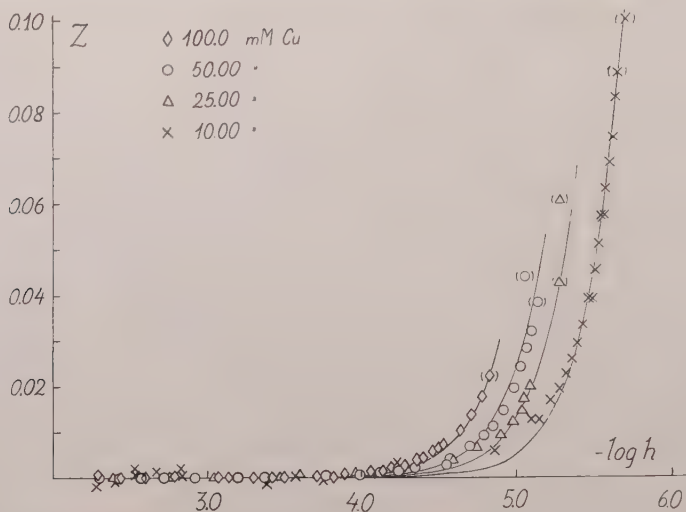
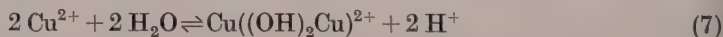


Fig. 3. Hydrolysis of Cu^{2+} . Z = average number of OH^- bound per Cu as a function of $(-\log h)$ (M scale). Points: experimental data. Curves calculated assuming that only the complex $\text{Cu}((\text{OH})_2\text{Cu})^{2+}$ is formed, with $k = 2.5 \times 10^{-11}$.

In Fig. 4, Z is plotted versus $x = \log B - 2 \log h$ for all values of B studied. The points are seen to lie on a single curve, indicating that the complexes present in the concentration range studied have the general formula $\text{Cu}((\text{OH})_2\text{Cu})_n^{2+}$ or $\text{Cu}(\text{OCu})_n^{2+}$. On the other hand, a large systematic trend was found when Z was plotted as a function of $\log B - \log h$ or of $\log B - 3 \log h$.

Then the values of $Z (=2y)$ were plotted as a function of $x = \log B - 2 \log h$ and compared with the " $y(X)$ " curves calculated by means of Sillén's equation ([10] ABII, equ. 22, 23). This is based on the assumption that only one complex is formed ("case II"). Good agreement was found between the experimental points and the calculated curve for $N=1$, corresponding to the complex $\text{Cu}((\text{OH})_2\text{Cu})^{2+}$. The logarithm of the equilibrium constant for the reaction



was found from the lateral translation $x - X$ required to make the experimental points coincide with the calculated curve. It was found that

$$\log k = -10.6 \pm 0.1.$$

Assuming this value, Z was calculated as a function of $\log h$ for $B=10.0$, 25.0, 50.0 and 100.0 mM; as is seen in Fig. 3 a satisfactory agreement with the experimental points was obtained.

Reasonable agreement was also obtained when the experimental curve $y(x)$ was compared with the curve $y(X)$ calculated assuming the unlimited mechanism "III a" [10] with $k_0=1-10$. Here the formation constant of the n th complex $k_n = k_0 k^n$ where k and k_0 are constants. It was found that $\log k_0 k = \log k_1 = -10.6 \pm 0.1$ in satisfactory agreement with the result obtained previously.

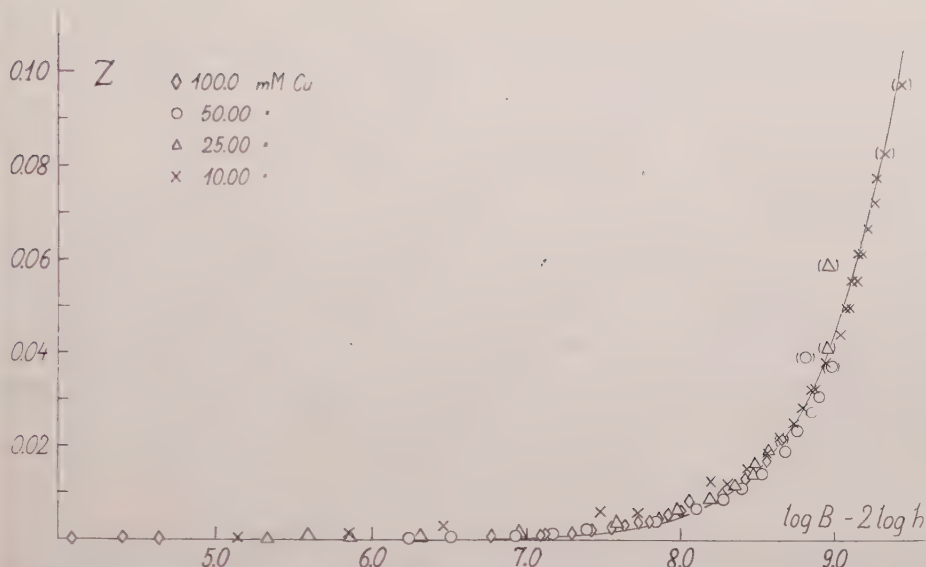


Fig. 4. Hydrolysis of Cu^{2+} . Z as a function of $\log B - 2 \log h$ (M scale). Points: experimental data. Curve calculated with $k = 2.5 \times 10^{-11}$.

With $k_0 = 1$	$\log k = -10.58$	$\log k_0 k = -10.58$
2	-10.90	-10.60
5	-11.30	-10.60
10	-11.62	-10.62

The fit was best for $k_0 = 5$ but the deviations even with $k_0 = 1, 2$ and 10 did not exceed the experimental error. The magnitude of k_0 indicates that for the low values of Z in question the main product of the hydrolysis is $\text{Cu}((\text{OH})_2\text{Cu})^{2+}$; the present data give no definite evidence for the existence of higher complexes such as $\text{Cu}_3(\text{OH})_4^{2+}$ etc.

Comparison with Pedersen's data [8]

From Pedersen's measurements on $\text{Cu}(\text{NO}_3)_2$ solutions Z may be calculated from the equation (with Pedersen's notation to the right)

$$Z = \frac{(\text{H}^+) + x + a\delta}{a} \quad (8)$$

where " (H^+) " = h (obtained from measurements with the glass electrode), " a " = B , " δ " is the ratio OH^-/Cu in his copper(II) nitrate, and " $x + a\delta$ " = $-H$ is the deficiency in the analytical concentration of acid. Fig. 5 shows Z plotted as a function of $(\log B - 2 \log h)$; the points for all values of B fall on a single curve except those for $B = 1.007$ and 2.005 mM which lie slightly above it. This indicates that the predominant products of hydrolysis have the general

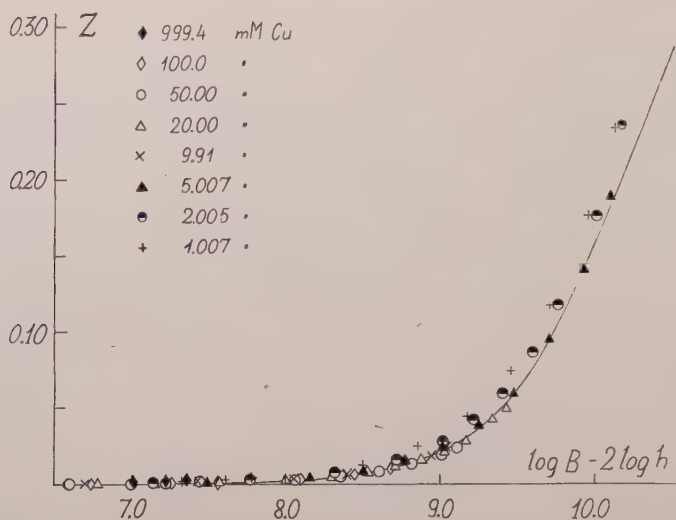
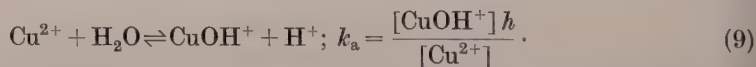


Fig. 5. Hydrolysis of Cu^{2+} . Z as a function of $\log B - 2 \log h$ (M scale). Points: experimental data of Pedersen [8]. Curve calculated assuming the formation of $\text{Cu}((\text{OH})_2\text{Cu})^{2+}$ only, with $k = 1.1 \times 10^{-11}$.

formula $\text{Cu}((\text{OH})_2\text{Cu})_n^{2+}$. Good agreement was obtained with curves calculated ([10] ABII, equ. 22, 23) assuming the formation of a single complex with $N=1$, i.e. $\text{Cu}((\text{OH})_2\text{Cu})^{2+}$. For the equilibrium represented by equ. (7) it was found that $\log k = -10.95 \pm 0.10$. Pedersen gave -10.89 for “ $\log K_3$ ” at infinite dilution.

Pedersen's experimental conditions (18°C , nitrate solutions, and varying ionic strength) were rather different from those used in this work. The fact that both sets of data give essentially the same result indicates that the hydrolysis reaction is but slightly influenced by the ionic medium.

The deviations of the points for $B=1.007$ and 2.005 mM from the calculated curve in Fig. 5 may be explained assuming the additional equilibrium



k_a may be computed according to [15]. From the equations:

$$\begin{aligned} BZ &= k_a [\text{Cu}^{2+}] h^{-1} + 2k [\text{Cu}^{2+}]^2 h^{-2} \\ B &= [\text{Cu}^{2+}] + k_a [\text{Cu}^{2+}] h^{-1} + 2k [\text{Cu}^{2+}]^2 h^{-2} \end{aligned}$$

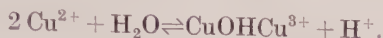
it follows that $[\text{Cu}^{2+}] = B(1-Z)$

$$\text{and} \quad k_a = hZ(1-Z)^{-1} - 2kB(1-Z)h^{-1} \quad (10)$$

From Pedersen's data for $B=1.007$ and 2.005 mM it was calculated that

$$k_a = (1.0 \pm 0.2) \cdot 10^{-8} \text{ M};$$

Pedersen's value ("K₁") is 1.07×10^{-8} M, extrapolated to infinite dilution. Pedersen claims the existence of a third complex, CuOHCu^{3+} , and gives "K₂" = 1.52×10^{-7} at zero ionic strength for the reaction



The complex $\text{Cu}_2\text{OH}^{3+}$, if it exists, will then have only a small influence on the *Z* values. To be strict, it should make the plot $Z (\log B - 2 \log h)$ deviate from a single curve, since it cannot be written in the "core + links" form, $\text{Cu}((\text{OH})_2\text{Cu})_n^{2+}$. In fact, when *Z* was calculated as a function of $\log B - 2 \log h$ assuming the two complexes $\text{Cu}_2\text{OH}^{3+}$ and $\text{Cu}_2(\text{OH})_2^{2+}$ and Pedersen's constants, the calculated deviations in an experimental range, $B = 10$ – 100 mM, were never greater than 0.001, thus within the experimental error. Pedersen's data cover a greater range in *B*; however, considering the uncertainty in the activity factors, it does not seem that the existence of $\text{Cu}_2\text{OH}^{3+}$ is as certain as that of $\text{Cu}_2(\text{OH})_2^{2+}$.

On the composition of the precipitate

In the last point of each series of measurements a solid phase was present. At the end of the titration, the light blue colored solid was collected on a sintered glass filter, washed with distilled water and acetone, dried at 25°C and weighed. Drying at 120°C did not change the weight. The solid was quantitatively dissolved from the filter in a known amount of HClO_4 . The solution was diluted to a definite volume, and aliquot portions of this stock solution were used for analysis.

Cu was measured iodometrically in a $\text{CH}_3\text{COONa} - \text{CH}_3\text{COOH}$ buffer mixture, in an atmosphere of CO_2 . This procedure yields accurate results within 1–2 %, as shown by the analysis of standard 0.01 M Cu-solutions.

The ClO_4^- content was estimated passing a definite volume of the stock solution through the hydrogen form of the ion exchanger Duolite C3 and titrating for the total H^+ in the eluate with 0.02 M NaOH.

Assuming the formula of the solid to be $\text{Cu}(\text{OH})_x(\text{ClO}_4)_{2-x}$, the mean value of *x* found from the Cu analysis of 3 different samples (47.2, 47.9 and 49.7 % Cu) was 1.60 ± 0.05 . From the ClO_4^- analysis of 2 samples, *x* was found to be 1.66 and 1.68. Since the weights of the samples analyzed were about 20–30 mg and the ClO_4^- content was a small difference between two large numbers, the last decimal in *x* cannot be considered significant. We shall write $\text{Cu}(\text{OH})_{1.6}(\text{ClO}_4)_{0.4}$ as the most probable formula of the solid phase.

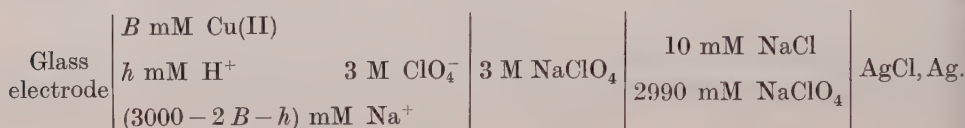
The X-ray powder photographs of three samples obtained from solutions of $\beta = 1.00$, 25.0 and 100.0 mM have been kindly examined by Dr. G. Lundgren and fil. mag. H. E. Forsberg. The samples gave identical X-ray patterns characteristic for a crystalline phase.

The basic salt formed from $\text{Cu}(\text{ClO}_4)_2$ solutions by the addition of NaOH has been previously investigated by Beebe and Goldwasser [16] and by Näsänen and Lamminen [17]. Beebe's and Goldwasser's analysis gave 3.5 for the ratio Cu/ClO_4^- in the solid, corresponding to $\text{Cu}(\text{OH})_{1.714}(\text{ClO}_4)_{0.286}$. According to Näsänen and Lamminen, $\text{Cu}(\text{OH})_2$ is formed from $\text{Cu}(\text{ClO}_4)_2$ solutions containing less than

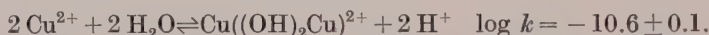
0.176 M excess ClO_4^- ; the solid is transformed to $\text{Cu}(\text{OH})_{1.714}(\text{ClO}_4)_{0.286}$ if the excess ClO_4^- is increased. To decide whether or not the small discrepancy between the formula proposed here and that of [16] and [17] is real, sufficient amounts of precipitate would have to be prepared under the various experimental conditions used in this work, [16] and [17] and analyzed very carefully.

Summary

The hydrolysis equilibria of the $\text{Cu}(\text{II})$ ion were investigated by measuring the emf of cells:



The range of B studied was 10–100 mM, and h varied from values where the hydrolysis is unappreciable until a precipitate was formed. The results may be explained assuming the equilibrium:



Copper amalgam electrodes could not be used to measure $[\text{Cu}^{2+}]$ in hydrolyzed solutions because of early precipitation of Cu_2O . Cu^{2+} was found to react with quinhydrone producing H^+ ions at $h < 1$ mM.

A potentiometric method is described for the accurate determination of the analytical hydrogen ion concentration in solutions containing hydrolyzing metal ions.

Pedersen's [8] measurements on $\text{Cu}(\text{NO}_3)_2$ solutions were analyzed by Sillén's [10] method. They are in satisfactory agreement with the results found in this work. From his data of $B = 1.005$ and 2.007 mM the equilibrium constant for the mononuclear step $\text{Cu}^{2+} + \text{H}_2\text{O} \rightleftharpoons \text{CuOH}^+ + \text{H}^+$ was estimated to be $(1.0 \pm 0.2) 10^{-8}$ M.

The crystalline phase formed from $\text{Cu}(\text{ClO}_4)_2$ solutions of $[\text{ClO}_4^-] = 3$ M by the addition of NaOH has the composition $\text{Cu}(\text{OH})_{1.60 \pm 0.05}(\text{ClO}_4)_{0.40 \pm 0.05}$.

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Umsatz der Trehalose in Bäckerhefe

I. Aufbau und Abbau der Trehalose bei der Glucosegärung

Von MAJKEN ELANDER

Mit 5 Figuren im Text

Trehalose kommt in Hefe vor, aber bis vor einigen Jahren herrschte die Auffassung, dass das Disaccharid Trehalose hauptsächlich in Bäckerhefe auftritt. Versuche, den Zucker in Bierhefe nachzuweisen, waren bis dahin ohne Erfolg geblieben (MÜNZ (1), TANRET (2), MYRBÄCK (3)). Im Jahre 1950 erschien jedoch eine Untersuchung von STEWART, RICHTMYER und HUDSON (4) über das Vorkommen von Trehalose in verschiedenen Hefen. Es wurde darin gezeigt, dass Trehalose ziemlich allgemein auch in Bierhefe, obschon in kleineren Mengen als in Bäckerhefe, vorkommt. PAYEN (5) fand, dass bei der Herstellung von aktiver Trockenhefe aus Bäckerhefe der Gehalt an Trehalose auf Kosten des Glykogens zunahm. POLLOCK und HOLMSTROM (6) konnten feststellen, dass der Trehalosegehalt in aktiver Trockenhefe während des Sprossens abnahm, während der Gehalt an Glykogen und Mannan beinahe unverändert blieb. Wie auch PAYEN (5) fanden sie, dass beim Trocknen der Gehalt an Trehalose in aktiver Trockenhefe zunahm. Wenn die Feuchtigkeit unter 50–45% herabsank, hörte die Zunahme auf.

BRANDT (7) konnte zeigen, dass, wenn Bäckerhefe unter Lüftung in Rohrzuckerlösung ohne Stickstoffquelle gezüchtet wurde, der Gehalt der Hefe an Trehalose bedeutend zunahm. Wurde die Hefe dagegen in Gegenwart von Ammoniumsulfat gezüchtet, nahm der Gehalt an Trehalose ab. Die Hefe zeigte im letzteren Fall ein lebhaftes Sprossen, was bei Züchtung ohne Stickstoff nicht der Fall war. Auch POLLOCK und HOLMSTROM (6) fanden, dass die Trehalose während des Sprossens der Hefe abgebaut wurde. Aus diesem Grund erscheint es wahrscheinlich, dass die Trehalose als Energiequelle der synthetischen Arbeit der Hefezelle eine wichtige Rolle spielt. Möglich ist, dass dem Disaccharid in dieser Hinsicht eine ähnliche Rolle zukommt wie dem Rohrzucker in grünen Pflanzen.

STEINER und CORI (8) zeigten, dass der Gehalt an Trehalose in frischer Bäckerhefe bei der Vergärung von Glucose erheblich zunahm. TREVELYAN *et al.* (9) analysierten den Gehalt an Glykogen, Mannan, Glucan und Trehalose vor und nach Vergärung von Glucose in frischer Bäckerhefe und fanden, dass hauptsächlich der Glykogengehalt bei der Gärung zunahm. Eine gewisse Zunahme von Mannan und Trehalose konnte jedoch auch nachgewiesen werden.

Neubildung von Trehalose bei Vergärung von Glucose mit Mazerationssaft aus Bierhefe ist von ELANDER und MYRBÄCK (10) und von NILSSON und ALM (11) unter-

sucht worden. Den Erstgenannten gelang es, das Disaccharid in kristallisierter Form zu isolieren. NILSSON und ALM fanden, dass die Synthese der Trehalose erst dann anfang, wenn die einleitende Phase der Phosphorylierung beendet war, und dass bei der Glucosegärung die Konzentration des Disaccharides beinahe geradlinig mit der Zeit zunahm. In diesen beiden Untersuchungen wurde etwa 20% der zugesetzten Glucose in Trehalose umgebaut.

In der vorliegenden Arbeit wird über fortgesetzte Untersuchungen der Trehalosebildung bei der Glucosegärung berichtet. Für diese Untersuchung war es wichtig, ein Hefepräparat herzustellen, das im Gegensatz zu Mazerationssaft wenigstens während einiger Monate haltbar war. NILSSON (12) hat darauf hingewiesen, dass der Gärungsverlauf in Mazerationssaft und in gewissen Trockenhefen grosse Ähnlichkeiten zeigt. Solche Trockenhefen sind Acetondauerhefe und luftgetrocknete Hefe in Gegenwart von Toluol und gewissen organischen Lösungsmitteln wie Benzol, Tetrachlorkohlenstoff und sek. Octylalkohol. Glucose wird nicht vollständig vergoren; Zwischen- bzw. Nebenprodukte werden angehäuft. Zusatz von Phosphat wirkt auf die Gärungsgeschwindigkeit stark aktivierend. In derartigen Hefepräparaten befinden sich die Enzymsysteme gewissermassen noch in ihrem ursprünglichen Milieu, so dass man mit grösserem Fug ihre Reaktionen mit denjenigen der frischen Hefe vergleichen kann, als das bei dem Mazerationssaft der Fall ist.

Es wurden Versuche ausgeführt, eine acetongetrocknete Hefe nach ALBERT, BUCHNER und RAPP (13) herzustellen. Diese Hefe zeigte sich als geeignetes Hefepräparat, weil es leicht herzustellen und beim Aufbewahren im Kühlschrank mehrere Monate haltbar war. Bei der Vergärung von Glucose wurde eine lebhaft Trehalosebildung hervorgerufen, die in mehreren Fällen diejenige in Mazerationssaft übertraf. Mit diesem Hefepräparat konnte eine genauere Untersuchung der Trehalosesynthese durchgeführt werden. Durch Bestimmungen von vergorener, d. h. in Kohlensäure und Alkohol zerlegter, veresterter und in Trehalose umgewandelter Glucose wurde versucht, festzustellen, ob die Totalmenge dieser Umsatzprodukte der zugesetzten Glucosemenge äquivalent war oder ob ein Teil der Glucose vielleicht in anderer Weise umgesetzt wurde.

In der früher veröffentlichten Untersuchung über Trehalosebildung in Mazerationssaft fanden ELANDER und MYRBÄCK (10), dass etwa 12% des organischen Phosphats in den Gärungsmischungen nicht als Bariumsalze mit 50%igem Alkohol bei pH 8,2 gefällt wurden. Sie vermuteten, dass das Esterphosphat wenigstens teilweise aus THP¹ bestehe und dass dieser Ester ein wahrscheinliches Zwischenprodukt bei der Trehalosesynthese sei. ROBINSON und MORGAN (14) haben nämlich früher gefunden, dass normalerweise bei der Vergärung von Glucose durch Acetondauerhefe eine Bildung von THP eintritt. Ebenso hat VEIBEL (15) gezeigt, dass THP bei Vergärung von Glucose durch frische Brauereiunterhefe in Gegenwart von Toluol gebildet wird. Diese Theorie wurde kürzlich durch eine kurze Mitteilung von LEOIR und CABIB (16) gestützt. Sie konnten in *Saccharomyces cerevisiae* ein Enzym nachweisen, dass die folgende Reaktion katalysiert: $\text{UDPG} + \text{G-6-P} \rightarrow \text{THP} + \text{UDP}$.

¹ Folgende Abkürzungen werden in diesem Aufsatz benutzt:

THP = Trehalosemonophosphorsäure
UDPG = Uridindiphosphoglucose
G-6-P = Glucose-6-phosphorsäure
UDP = Uridindiphosphorsäure
DPN = Diphosphopyridinnucleotid
HDP = Hexosediphosphorsäure

HMP = Hexosemonophosphorsäure
G-1-P = Glucose-1-phosphorsäure
F-6-P = Fructose-6-phosphorsäure
F-1,6-P = Fructose-1,6-diphosphorsäure
ATP = Adenosintriphosphorsäure
ADP = Adenosindiphosphorsäure

In dieser Untersuchung sind Versuche angestellt worden, die Bildung von THP in denselben Gärmischungen analytisch nachzuweisen, in denen die Konzentration von Trehalose bestimmt worden war.

Schliesslich wurde die Einwirkung von Arsenat auf die Bildung von Trehalose untersucht. SUSSMAN und SPIEGELMAN (17) haben gezeigt, dass Arsenat gewisse, energieverlangende Prozesse hemmt. Es war deswegen von Interesse, herauszufinden, ob durch Arsenat eine Hemmung der Trehalosesynthese in der acetonetrockneten Hefe hervorgerufen wurde, was wiederum die Bedeutung der Bildung von energiereichem Phosphat für die betreffende Synthese zeigen würde.

Experimenteller Teil

Herstellung von Hefepräparaten

Acetonetrocknete Hefe wurde aus frischer Bäckerhefe von Stockholms Norra Jästfabrik hergestellt. Die Hefe wurde nach ALBERT, BUCHNER und RAPP (13) mit Aceton und Äther behandelt. Das Trocknen wurde jedoch bei Zimmertemperatur im Vakuumexsiccator über Blaugel statt bei 45° ausgeführt. Dadurch wurde die Hefe vollständiger von Aceton befreit, was höhere Gärungsaktivität und bessere Haltbarkeit mit sich brachte. Die Feuchtigkeit der acetonetrockneten Hefe machte etwa 7% des Trockengewichtes aus.

Die schwedische Presshefe enthält wechselnde Mengen von Trehalose, die durch die Behandlung mit Aceton und Äther nicht entfernt werden. Nach dem Trocknen kann die Trehalose aus der Hefe mit Wasser leicht extrahiert werden.

Die Hefetrehalose wurde in folgender Weise bestimmt: 1 g Acetonhefe wurde 5 Min. mit 10 ml dest. Wasser geschüttelt, wonach die Hefe abzentrifugiert wurde. Dies Verfahren wurde dreimal wiederholt und die Trehalose danach in den vereinigten Zentrifugaten bestimmt.

Trehalosefreie Acetonhefe, im folgenden „gewaschene Hefe“ genannt, wurde folgendermassen hergestellt: 25 g Acetonhefe wurde mit 900 ml dest. Wasser, 50 ml 1 M Phosphatpuffer (pH 6,4) und 20 ml 1 M Magnesiumchlorid ausgerührt. Die Hefe wurde bei +3° abzentrifugiert. Das Zentrifugat wurde entfernt und dasselbe Verfahren noch einmal wiederholt. Die Hefe wurde dann in 150 ml dest. Wasser von 0° suspendiert, wieder abzentrifugiert und nach NILSSON (18) so schnell wie möglich in dünnen Schichten auf Tonplatten getrocknet. 25 g Acetonhefe geben etwa 15 g gewaschene Hefe, mit einer Feuchtigkeit von etwa 9%. Die Gärungsfermente wurden durch das Trocknen teilweise inaktiviert. Zu den Gärproben musste deswegen von der gewaschenen Hefe 50% mehr als von der acetonetrockneten genommen werden. Die gewaschene Hefe war erheblich haltbarer als die Acetonhefe. Beim Aufbewahren im Kühlschrank mehr als zwei Jahre lang konnte nur eine unbedeutende Abnahme des Gärungsvermögens beobachtet werden. Die Acetondauerhefe war nur 3–4 Monate brauchbar.

Gärungsmethodik

Die Gärungsversuche wurden nach dem Verfahren von MYRBÄCK (19) ausgeführt. Die Gärproben wurden im Thermostat bei 30° geschüttelt. Die entwickelte Kohlendioxidmenge wurde in Gasbüretten über Quecksilber gemessen. Die Gärungen wurden dadurch unterbrochen, dass die Proben schnell in Eiswasser abgekühlt

wurden, wonach die Acetonhefe abzentrifugiert wurde. In den meisten Versuchen wurde noch vorhandene freie Glucose durch Vergärung mit frischer Presshefe aus den Zentrifugaten entfernt. Die frische Hefe greift in mässigen Versuchszeiten weder Zuckerphosphorsäureester noch Trehalose an. Für eine kleine Gärung der Trehalose kann eine Korrektur leicht angebracht werden.

Als Fermentsystem bei den Gärungen ist sowohl acetongetrocknete als auch gewaschene Hefe angewandt worden. Die acetongetrocknete Hefe brauchte nur mit Magnesiumionen und Phosphat ergänzt zu werden, um maximales Gärvermögen zu entwickeln. Die gewaschene, trehalosefreie Acetonhefe zeigte kein Gärungsvermögen, weil zusammen mit der Trehalose auch DPN entfernt wurde. Es wurden Versuche unternommen, das Fermentsystem mit einem Cozymasepräparat zu ergänzen, das nach SUMNER (20) dargestellt war. Das Gärungsvermögen in einem System von gewaschener Hefe und Cozymase war jedoch bedeutend niedriger, als wenn man nur die acetongetrocknete Hefe verwendete. Dies deutete darauf hin, dass ausser DPN, auch andere für die Gärung wichtige Stoffe, beim Waschen entfernt wurden. Mit Kochsaft aus luftgetrockneter Brauereiunterhefe nach NILSSON (18) konnte das Gärungsvermögen vollständig wiederhergestellt werden. Da es sich herausstellte, dass die Brauereiunterhefe meistens nur kleine Mengen von Trehalose enthielt, wurde durchgehend Kochsaft aus derartiger Hefe als Ergänzung für die gewaschene Hefe verwendet.

Die Gärungsversuche, in welchen Trehalose bestimmt wurde, wurden entweder in Gärröhrchen (2 × 9 cm) oder in Erlenmeyerkolben von 100 ml ausgeführt. In beiden Fällen wurde die Kohlensäureentwicklung volumetrisch verfolgt. Wenn nichts anderes angegeben ist, hatten die Gärproben folgende Zusammensetzung: 200 mg Acetonhefe, 100 mg Glucose, 0,1 ml 0,2 *M* Magnesiumchlorid, 0,1 ml 1 *M* Phosphatpuffer (pH 6,4), 0,04 ml 2%ige Acetaldehydlösung und dest. Wasser ad 2 ml. Mit gewaschener Hefe war die Zusammensetzung der Proben die folgende: 300 mg gewaschene Hefe, 100 mg Glucose, 0,1 ml 0,2 *M* Magnesiumchlorid, 0,1 ml 1 *M* Phosphatpuffer, (pH 6,4), 0,5 ml Hefekochsaft (ein Teil trockener Unterhefe und 3 Teile Wasser), 0,03 ml HDP-Lösung (13,1 mg org. Phosphat/ml), 0,04 ml 2%ige Acetaldehydlösung und dest. Wasser ad 2 ml.

Enteiweissung der Gärproben

In den Gärproben, in welchen Zuckerbestimmungen ausgeführt werden sollten, sind die Proteine nach zwei verschiedenen Methoden entfernt worden. Nach der Methode von WEST, SCHARLES und PETERSON (21) wurden die Proteine mit 30%igem Quecksilbersulfat in 10%iger Schwefelsäure gefällt, wonach mit festem Bariumkarbonat neutralisiert wurde. Aus den Gärproben wurde die Hefe zunächst durch Zentrifugieren abgetrennt. Nach Verdünnen mit dem gleichen Volumen Wasser wurden 0,55 ml Quecksilbersulfat pro Gärprobe zugesetzt; im Falle gewaschener Acetondauerhefe 1 ml. Zusammen mit den Proteinen wurden auch anorganisches Phosphat und die Hauptmenge des HDPs durch diese Methode entfernt. Nach der von SOMOGYI (22) beschriebenen Methode wurden die Proteine mit Bariumhydroxyd und Zinksulfat gefällt. Die Gärproben wurden wie oben verdünnt. 1 ml der verdünnten Probe wurde mit 5 ml von jedem Reagens gefällt. Zusammen mit anorganischem Phosphat und HDP wurde auch ein Teil des HMPs durch diese Methode entfernt. Dies Verfahren war erheblich einfacher als die Quecksilbersulfatmethode, es hatte aber den

Nachteil, dass die Verdünnung der Proben für die direkte Bestimmung der optischen Aktivität zu gross war.

In den Proben, in welchen organisches Gesamtphosphat bestimmt werden sollte, wurden die Proteine mit Trichloressigsäure entfernt. Es wurde so viel 30%ige Trichloressigsäure zugesetzt, bis eine Endkonzentration von 5% erreicht wurde.

Bestimmung der Trehalose

Die Trehalose wurde aus der Zunahme von reduzierender Substanz nach Säurehydrolyse und, wenn dies möglich war, aus der optischen Aktivität bestimmt. Neugebildete Trehalose wurde aus dem Unterschied zwischen dem Gehalt an Trehalose in den Gärproben und in einem Wassereextrakt der Acetonhefe festgestellt.

Die Reduktionsbestimmungen wurden nach SOMOGYI (23) ausgeführt. Die Konzentration der Trehalose wurde aus dem Unterschied zwischen dem Reduktionswert nach fünfständiger Hydrolyse in 1 *N* Schwefelsäure im siedenden Wasserbad und dem direkt bestimmten Reduktionswert erhalten. Unter diesen Bedingungen wurde etwa 80% der Trehalose hydrolysiert. Der Hydrolysegrad schwankte jedoch etwas vom einem Versuch zum anderen, weswegen es notwendig war, gleichzeitig mit den Gärproben Standardproben von Trehalose zu hydrolysieren. Ein Korrektionsfaktor der unvollständigen Hydrolyse konnte dadurch eingeführt werden.

In der Hefezelle kommen drei Polysaccharide in ähnlichen Konzentrationen wie die der Trehalose vor, nämlich Glykogen, Mannan und Glucan. Durch Digerieren der Hefe mit 30%iger Kalilauge bei 100° fand TREVELYAN (24), dass das Mannan und ein Teil des Glykogens herausgelöst wurden. Durch Behandlung des Rückstandes mit 60%iger Schwefelsäure bei Zimmertemperatur lösten sich der Rest des Glykogens und das Glucan heraus. Glucan, das als Stützsubstanz der Zellmembran dient, ist fest an unlöslichen Zellbestandteilen verankert. Es ist deswegen kaum zu befürchten, dass dieses Polysaccharid während der Acetonhefegärungen teilweise in Lösung geht. Das Vorkommen in den Gärmischungen von Glykogen und Mannan, die sich beträchtlich leichter als Glucan lösen, war dagegen nicht unwahrscheinlich und wurde untersucht. Nach LEPAGE (25) wird Glykogen mit 50%igem Alkohol ausgefällt. Nach Enteiweissen mit 5%iger Trichloressigsäure wurden die Gärproben mit dem gleichen Volumen Alkohol versetzt; dabei entstand eine kleine Trübung, die abfiltriert und getrocknet wurde. Aus 9 g gewaschener Acetonhefe wurde einmal 45 mg und ein anderes Mal 42 mg Niederschlag erhalten. Der Niederschlag wurde in wenig Wasser gelöst und auf Glykogen mit Jodlösung geprüft. Da die Probe dabei nicht gefärbt wurde, konnte der Niederschlag nicht aus Glykogen bestehen. Die Probe wurde dagegen bei Zusatz von Fehlings Lösung deutlich getrübt, was auf das Vorkommen von Mannan hindeutete. Da aber in den Proben, in welchen die Zuckerbestimmungen ausgeführt wurden, keine Trübung mit Fehlings Lösung entstand, muss der Gehalt an Mannan äusserst gering gewesen sein.

Es ist klar, dass, wenn die Bestimmungen der Trehalose aus der Zunahme des Reduktionswertes zuverlässig sein sollen, keine Änderung des direkt bestimmten Reduktionswertes entstehen darf; weiter dürfen keine anderen Substanzen in den Gärproben vorkommen, die bei der Hydrolyse grösseren Reduktionswert bekommen. Der Reduktionswert der freien Glucose veränderte sich während fünfständiger Säurehydrolyse nicht, was aber bei den Phosphorsäureestern der Fall war. Analysen ihrer Zusammensetzung, die später publiziert werden sollen, haben gezeigt, dass man sicher mit den folgenden Estern rechnen musste: G-1-P, G-6-P, F-6-P und

F-1,6-P und wahrscheinlich auch mit THP. G-1-P gibt wie Trehalose reduzierenden Zucker bei der Säurehydrolyse. Beim Gegenwart dieses Esters werden deswegen die Trehalosewerte zu hoch. Die Zunahme des Reduktionsvermögens des G-6-Ps bei der Säurehydrolyse ist so unbedeutend, dass man damit nicht zu rechnen braucht. Kommt THP vor, wird dieser Ester zusammen mit der Trehalose in den Proteinfiltraten bestimmt. Die Fructoseester werden bei der Hydrolyse teilweise zu nicht reduzierenden Substanzen abgebaut. Die Trehalosewerte werden deswegen von ihnen herabgesetzt. Die Einwirkung der Hexosemonophosphorsäureester auf die Bestimmungen der Trehalose wird demnach von ihrer Menge und Zusammensetzung abhängig sein. Durch Ausfällen mit Bariumacetat und 70-80%igem Alkohol bei pH 8,2 nach LEPAGE (25) sind sie in mehreren Versuchen entfernt worden, wodurch man sich eine Auffassung über ihre Einwirkung auf die Trehalosewerte bei verschiedenen Gärzeiten bilden konnte.

Nachdem die freie Glucose mit Presshefe und die Proteine mit Quecksilbersulfat entfernt worden waren, konnte die Trehalose in mehreren Versuchen aus der optischen Aktivität der Lösungen direkt bestimmt werden. Die Proteinfiltrate waren jedoch oft von kolloidalem Quecksilber dunkel gefärbt, was die optischen Bestimmungen unzuverlässig machte. Für Trehalose mit 2 Kristallwassern ist nach SCHUKOW (26) $[\alpha]_D^{20} = +178,3^\circ$. War freie Glucose zugegen, konnte eine annähernde Korrektur ihrer Drehung gemacht werden, $[\alpha]_D^{20}$ für Glucose ist $+52,3^\circ$. Auch die Phosphorsäureester wirken auf die optische Aktivität der Proben ein. Für sie konnte jedoch keine Korrektur eingeführt werden, da ihre Zusammensetzung nicht bekannt war.

Bestimmung der Phosphorsäure

Die Phosphorsäure wurde nach der spektrophotometrischen Methode TEORELLS (27) bestimmt, die nur wenig von der nach FISKE SUBBAROW abweicht. Die erstgenannte hat jedoch den Vorteil, dass G-1-P während der Zeit der Messungen vom Reagens nicht hydrolysiert wird.

Die Veraschungen wurden mit halbkonzentrierter Schwefelsäure und einigen Tropfen 30%igen Wasserstoffsuperoxyds ausgeführt. Die Adsorption wurde bei 720 m μ in Beckman Spektrophotometer Modell DU gemessen. Geeigneter Messungsbezirk war 10–150 γ Phosphor.

Herstellung kristallisierter Trehalose

Wie einleitungsweise hervorgehoben, ist Trehalose von ELANDER und MYRBÄCK (10) aus Gärmischungen mit Mazerationssaft isoliert worden. Dieselbe Methodik wurde auch auf mit Acetonhefe vergorene Proben geprüft, es wurden jedoch nur klebrige, öltartige Substanzen erhalten. Die Proben waren gelb gefärbt. Die gelbe Farbe konnte jedoch durch Kochen mit Knochenkohle und Filtrieren durch Kieselgur entfernt werden. STEWART und Mitarbeiter (4) fanden, dass die Kristallisation der Trehalose aus Hefeextrakten erleichtert wurde, wenn die Proben den Kationenaustauscher IR-100 und den Anionenaustauscher IR-4B durchliefen. Diese Reinigung wurde geprüft und führte zu guten Ergebnissen. Das aktive Volumen des Ionenaustauschers war dabei 15 cm $\times$ 3 cm². Das Volumen der Proben war 50–125 ml. Die Konzentration des Esterphosphates wurde durch die Passage des Anionenaustauschers um etwa 10% erniedrigt. Die Ninhydrinreaktion, die vorher positiv war, wurde nachher negativ. Nach der Behandlung mit Ionenaustauschern wurden die

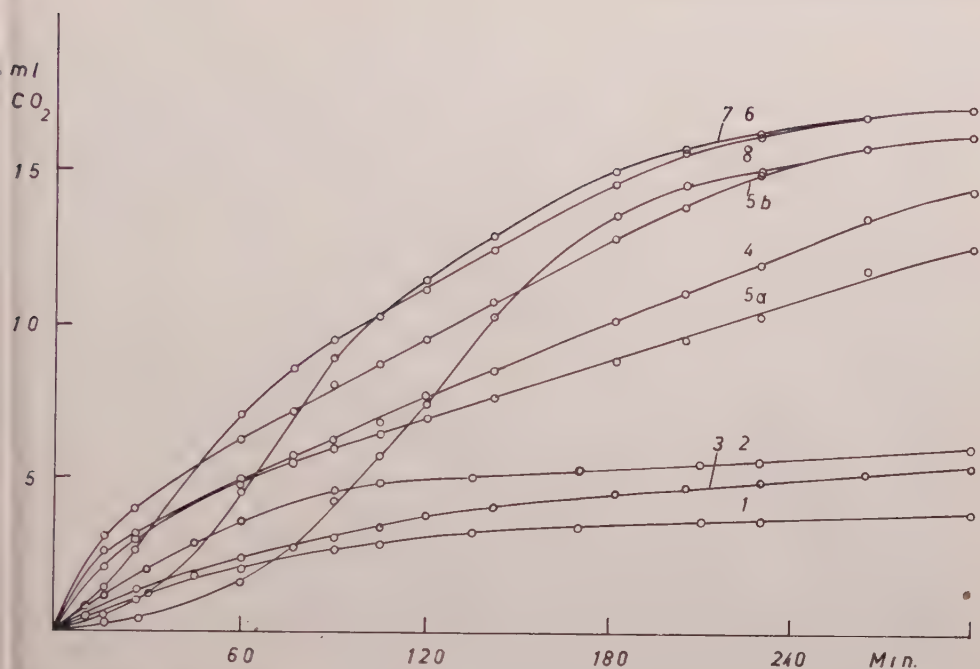


Fig. 1. Gärung mit Acetondauerhefe bei verschiedenen Phosphatkonzentrationen. Die Zusammensetzung der Gärproben ist im experimentellen Teile angegeben. Zu Probe 5a wurde jedoch kein $MgCl_2$ und zu den Proben 1 und 2 keine Glucose zugesetzt.

- | | |
|---------------------------------|-------------------------------------|
| 1. Ohne Zusatz von Phosphat. | 5a u. b Zusatz von 0,05 M Phosphat. |
| 2. Zusatz von 0,05 M Phosphat. | 6. » » 0,10 M » |
| 3. Ohne Zusatz von Phosphat. | 7. » » 0,15 M » |
| 4. Zusatz von 0,025 M Phosphat. | 8. » » 0,20 M » |

Proben bis zur Sirupkonsistenz eingeengt und mit Alkohol bis zu 85% versetzt. Beim Zusatz des Alkohols fielen mehrmals flockige Niederschläge aus, die abfiltriert werden mussten. Im Kühlschrank kristallisierte nach einiger Zeit die Trehalose aus.

Versuchsergebnisse

I. Glucosegärung mit Acetondauerhefe

Der Verlauf der Glucosegärung durch Acetondauerhefe bei verschiedenen Phosphatkonzentrationen wird in Fig. 1 dargestellt. Die Kurven 1 und 2 zeigen die Selbstgärung der Hefe ohne und mit Phosphat. Die aktivierende Wirkung von Magnesiumion geht aus dem Vergleich der Kurven 5a und 5b hervor. Fig. 2 stellt die Glucosegärung der gewaschenen Hefe bei Zusatz von Hefekochsaft und verschiedenen Phosphatkonzentrationen dar. Die Kurven 1 und 2 in Fig. 2 zeigen die Selbstgärung dieses Enzymsystemes. Die stärkere Selbstgärung der gewaschenen Dauerhefe im Vergleich zu der nicht gewaschenen Acetondauerhefe beruht nur darauf, dass von der ersteren 50% mehr zugesetzt wurde. Durch Vergleich zwischen Fig. 1 und Fig. 2 geht hervor, dass der Gärungsverlauf durch das Auswaschen nicht verändert wird.

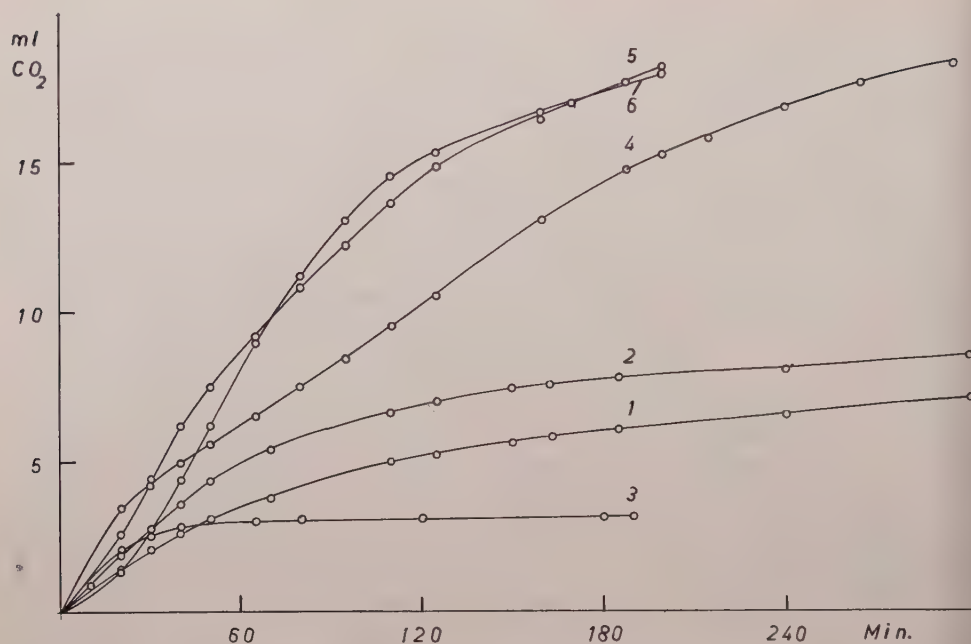


Fig. 2. Gärung mit gewaschener Acetondauerhefe bei verschiedenen Phosphatkonzentrationen. Zu den Proben 1 und 2 wurde keine Glucose zugesetzt, zu den übrigen 100 mg pro Gärprobe à 2 ml.

- | | |
|--------------------------------|--------------------------------|
| 1. Ohne Zusatz von Phosphat. | 4. Zusatz von 0,05 M Phosphat. |
| 2. Zusatz von 0,05 M Phosphat. | 5. » » 0,10 M » |
| 3. » » 0,025 M » | 6. » » 0,15 M » |

Ohne Zusatz von Phosphat wurde Glucose äusserst langsam vergoren (Fig. 1, Kurve 3). Zusatz von Phosphat steigerte die Gärgeschwindigkeit. Wurde mehr als 0,05 M Phosphat zugesetzt, trat zu Anfang der Gärung eine Induktionsperiode auf (Fig. 1, Kurve 4–8, Fig. 2, Kurve 3–6).

Der Gärungsverlauf stimmte hauptsächlich mit dem Gärungsverlauf überein, den NILSSON (12) als charakteristisch für acetonegetrocknete Unterhefe gefunden hat. Die Vergärung der zugesetzten Glucose war unvollständig, Zusatz von Phosphat rief eine Differenzierung des Gärungsverlaufes hervor. In einigen Hinsichten unterschied sich jedoch die Bäckerhefe von der Brauereiunterhefe: ohne Zusatz von Phosphat war die Gärung unbedeutend, die Gärung wurde durch Magnesiumionen aktiviert, die durch Phosphat hervorgerufenen Induktionsperioden waren länger.

2. Zuwachs- und Färbungsversuche mit Acetondauerhefe

Da alle Gärversuche dieser Untersuchung, mit einer Ausnahme, an der Luft ausgeführt wurden, war es von Interesse, darüber Auskunft zu erhalten, ob die Zahl der zuwachs-fähigen Zellen der Acetondauerhefe so gross war, dass ihr Stoffwechsel auf die Zusammensetzung der Gärprodukte, vor allem die Bildung der Trehalose, einwirken konnte.

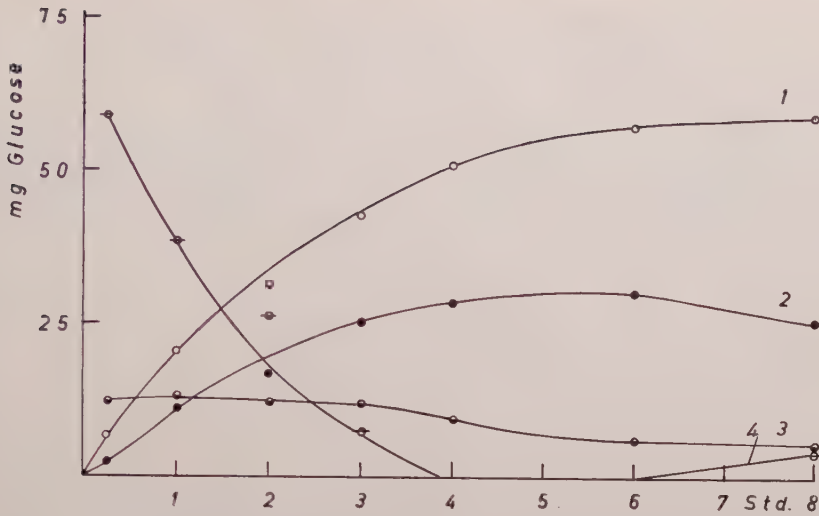


Fig. 3. Umsatzprodukte bei der Acetonhefegärung.

1. ○ — ○ vergorene Glucose. 3. ● — ● veresterte Glucose.
 2. ● — ● neugebildete Trehalose. 4. ○ — ○ freie Glucose.

Die Zuwachsversuche wurden teils in Nährlösung, teils auf Würzeagar ausgeführt. Die Nährlösung wurde aus 2%iger Glucose und Hefekochsaft hergestellt. 12 sterile Reagenzgläser mit Nährlösung wurden vorbereitet. Von ihnen wurden fünf mit einer Suspension aus Acetondauerhefe (1 g in 10 ml Wasser) und fünf mit einer Suspension gewaschener Hefe derselben Konzentration mit einer Platinöse geimpft. Zwei Gläser wurden nicht geimpft sondern dienten als Null-Proben. Die Gläser wurden im Thermostat bei 30° gehalten. Am folgenden Tage konnte nur eine schwache Trübung in den geimpften Gläsern beobachtet werden. Nach weiteren 24 Stunden war die Trübung ganz deutlich geworden.

10 sterile Platten mit Würzeagar wurden hergestellt. Die Konzentration der Hefesuspensionen, mit denen die Platten geimpft wurden, entsprach der von BARTHEL, EULER und MYRBÄCK (28) angegebenen. Diese Autoren untersuchten den Zuwachs auf Würzeagar von Brauereitrockenhefe nach Behandlung mit Alkohol, Äther und Chloroform. 1 g Hefe wurde in 75 ml Wasser suspendiert, 1 ml davon wurde bis auf 100 ml verdünnt. Folgende Suspensionen wurden geprüft: frische Bäckerhefe, Acetondauerhefe, gewaschene Acetondauerhefe, ferner letztere nach 4 Stunden Gärung in Glucoselösung. Mit jeder Suspension wurden zwei Platten geimpft. Die eine Hälfte der Platte wurde mit einer Platinöse, die andere mit einer sterilen Pipette geimpft. Die Platten wurden ins Konstantzimmer bei 23° hineingestellt. Nach 6 Tagen hatten sich im Falle der frischen Bäckerhefe zahlreiche Kolonien entwickelt. Bei der Acetondauerhefe fanden sich nur vereinzelte Kolonien auf den mit der Pipette geimpften Plattenhälften. Acetondauerhefe, die zuerst 4 Stunden in Glucoselösung gegoren hatte, zeigte keine gesteigerte Entwicklung von Kolonien.

Es wurden ferner einige Färbungsversuche mit acetongetrockneten Hefezellen ausgeführt. Die Zellen wurden mit Methylenblau im Phosphatpuffer, pH 4,6, nach FINK und KÜHLES (29) gefärbt. Diese Farbflüssigkeit dringt nur langsam in lebende

Tab. 1. Vergleich der Trehalosebildung in Luft und in Stickstoff.

Alle Werte in mg Glucose pro Gärprobe berechnet.

Nr.	Gärzeit, Min.	Mit Acetondauerhefe vergorene Glucose	Mit Bäckerhefe vergorene Glucose	Reduzierende Substanzen		Diff.	Hydrolysegrad der Trehalose. in %	Nach Korr. unvollst. Hydrolyse	Trehalose aus opt. Aktivität berechn.
				vor Hydrolyse	nach Hydrolyse				
1				4,5	15,8	11,3	73,5	15,4	16,7
2				3,7	15,5	11,8	»	16,1	16,7
3	270	52,3	5,8	4,2	35,5	31,3	79,0	39,6	40,4
4	»	52,3	5,6	4,0	35,3	31,3	»	39,6	40,4
5	270	52,3	8,6	3,7	34,8	31,1	79,1	39,3	39,1
6	»	52,3	9,7	3,6	33,8	30,2	»	38,2	38,6

Zellen ein. Die Färbbarkeit der frischen und der acetonetrockneten Hefezellen vor und nach dem Zuwachs in Nährlösung während dreier Tage bei 30° wurde verglichen. Die frischen Zellen wurden nicht gefärbt, in die acetonetrockneten drang die Farblösung sofort ein. Letztere waren gegenüber den frischen Zellen bedeutend kleiner und ihre Form war unregelmässig, während die frischen oval und glatt aussahen. Nach Züchtung der Acetondauerhefe konnten sowohl gefärbte als auch ungefärbte Zellen beobachtet werden. Die ungefärbten Zellen waren den frischen in Grösse und Form ähnlich. Zahlreiche Zellverbände aus 2 bis 3 Zellen bestehend, wurden gesehen, die Zellen waren entweder alle ungefärbt oder die Verbände bestanden aus einer ungefärbten und einer gefärbten Zelle.

Da, wie die Versuche zeigen, der Zuwachs in den in Frage kommenden Versuchszeiten nur ganz unbedeutend ist, erscheint es unwahrscheinlich, dass die wenigen reproduktionsfähigen Zellen irgendwelchen Einfluss auf die Zusammensetzung der Gärprodukte haben können.

3. Umsatz der Trehalose in Gegenwart von 0,05 M Phosphat

a) Mit Acetondauerhefe als Fermentsystem

Vergleich zwischen Trehalosebildung in Luft und in Stickstoff

Die Gärmethodik, die bei dieser Untersuchung verwendet wurde, wird in hohem Grade vereinfacht, wenn in Luft gearbeitet werden kann. Die Zuwachsversuche zeigten, dass eine gewisse Anzahl Zellen der Acetondauerhefe das Reproduktionsvermögen auch nach der Behandlung mit Aceton und Äther behielten. Der langsame Zuwachs der Zellen deutete aber darauf hin, dass die Anzahl der reproduktionsfähigen Zellen klein war. Ob diese Zellen das Vermögen besaßen, Glucose aerob abzubauen, konnte durch vergleichende Atmungs- und Gärungsversuche geklärt werden. Derartige Bestimmungen gehörten jedoch nicht zum Ziele dieser Untersuchung, wobei als wesentlich nur herauszufinden war, ob die Gegenwart von Sauerstoff auf das Vermögen der Acetondauerhefe, Trehalose aufzubauen, einwirkte. Hierüber wurde durch Vergleich der Trehalosebildung in Luft und in Stickstoff Auskunft erhalten.

Tab. 2. Umsatz der Trehalose mit Acetondauerhefe.

Alle Werte als mg Glucose pro Gärprobe berechnet.

Nr.	Gärzeit, Min.	Vergorene Glucose	Reduzierende Substanzen		Diff.	Hydrolysegrad der Trehalose in %	Nach Korr. unvollst. Hydrolyse	Trehalose aus opt. Aktivität bestimmt	Neugebildete Trehalose in % vergorener Glucose
			vor Hydrolyse	nach Hydrolyse					
1			3,4	14,2	10,8	73,0	14,8	14,9	
2a	300	60,0	2,1	45,1	43,0	85,2	50,5	50,8	
2b*			0,9	44,2	43,3	86,2	50,2	50,3	59,5
3	310	4,1	99,5	110,5	11,0	84,8	13,0		
4			4,3	15,8	11,5	73,2	15,7	16,4	
5			4,4	16,4	12,0	»	16,4	16,7	
6	15	6,4	62,2	76,7	14,5	79,4	18,3		34,4
7	60	20,6	42,8	64,4	21,6	»	27,2		53,9
8	120	31,4	30,2	56,6	26,4	79,5	33,2		54,5
9	180	43,1	11,4	44,2	32,8	»	41,3		58,4
10	240	51,3	2,2	37,8	35,6	»	44,8		56,0
11	360	58,0	2,2	39,3	37,1	»	46,7		52,8
12	480	59,5	6,8	40,2	33,4	»	42,0		43,5
13	1 380	66,4	37,7	41,6	3,9	»	4,9		
14	180	39,2	21,0	50,8	29,8	75,2	39,6		59,9

* Nach Ausfällen des HMPs.

Vier gleichlaufende Gärungsversuche wurden angestellt, von denen zwei in Luft und zwei in Stickstoff ausgeführt wurden. Der handelsübliche Stickstoff enthält gewöhnlicherweise 0,5–1 % Sauerstoff. Um diesen zu entfernen, wurde das Gas durch zwei Waschflaschen mit Pyrogallol in Kalilauge hindurchgeleitet (30) (15 g Pyrogallol in 15 ml Wasser und 135 ml gesättigter Kalilauge). Das gewaschene Gas durfte dann durch die wohl abgekühlten Gärproben während 40 Min. hindurchbrodeln. Das Volumen der Proben war 20 ml. Die Gärversuche wurden durch Abkühlen unterbrochen, die Acetonhefe abzentrifugiert und die freie Glucose der Proben mit Bäckerhefe vergoren. Die Proben wurden danach mit Quecksilbersulfat enteiweisst und die Trehalose in den Proteinfiltraten aus der Zunahme des Reduktionswertes und aus der optischen Aktivität bestimmt.

Die Versuchsergebnisse werden in Tab. 1 dargestellt. Die Proben 1 und 2 zeigen den Gehalt an Trehalose der Acetondauerhefe. In Proben 3 und 4 ist der Trehalosegehalt nach Vergärung in Luft und in Proben 5 und 6 nach Vergärung in Stickstoff bestimmt. Aus Tab. 1 geht hervor, dass eine bedeutende Neubildung von Trehalose in der Acetondauerhefe bei der Glucosegärung stattfand. Die Trehalosewerte der beiden Analysemethoden stimmten sehr gut überein. Die in Trehalose umgewandelte Glucose betrug etwa 45% der gleichzeitig vergorenen. In der Gärgeschwindigkeit zwischen den Proben, die in Luft und in Stickstoff ausgeführt waren, konnte kein Unterschied beobachtet werden. Die Neubildung der Trehalose war in der einen Stickstoffprobe etwas niedriger als in den übrigen, weshalb der Mittelwert der Stickstoffproben etwa 3,5% niedriger als der der Luftproben ist. Ob der Unterschied zufällig war oder mit der Zusammensetzung des bases während der Gärung zusammenhängen konnte, lässt sich nicht sagen. Der Unterschied war auf alle Fälle so klein, dass die Ausführung der Versuche in Luft durchaus zulässig erschien.

Tab. 3. Verteilung der Esterphosphate bei der Gärung mit Acetondauerhefe.
3,36 mg anorganisches Phosphat wurde pro Gärprobe à 2 ml zugesetzt. Glucose und Phosphat in mg berechnet.

Nr.	Gärzeit, Min.	Trichloressigsäurefiltrate			HgSO ₄ - filtr. Org. Phosphat	Mit HDP äquiv. Glucose	Mit HMP äquiv. Glucose	Umsatzpro- dukte in % zuges. Glu- cose
		Gesamt- phosphat	Anorg. Phosphat	Org. Phosphat				
4		1,27	0,50	0,77				
5		1,25	0,48	0,77				
6	15	4,57	1,15	3,42	0,78	7,7	4,5	79,6
7	60	4,64	1,07	3,57	0,96	7,6	5,6	83,5
8	120	4,63	1,33	3,30	0,88	7,0	5,1	87,0
9	180	4,63	1,39	3,24	0,90	6,8	5,2	87,8
10	240	4,72	2,21	2,51	0,77	5,0	4,5	91,7
11	360	4,74	3,29	1,45	0,64	2,4	3,7	96,9
12	480	4,75	3,47	1,28	0,65	1,8	3,8	94,9
13	1 380	5,03	3,96	1,07	0,50	1,7	2,9	95,3
14	180	4,88	1,14	3,74	1,03	7,9	6,0	93,1

Verfolgung des Umsatzes der Trehalose während 23 Stunden

In Tab. 2 werden die Ergebnisse zweier Versuche über den Umsatz der Trehalose in Acetondauerhefe bei der Vergärung von Glucose gezeigt. Die freie Glucose wurde in diesen Versuchen nicht beseitigt; deswegen betragen die Reduktionswerte die Summe der Reduktion der freien Glucose und der reduzierenden Phosphorsäureester. Die Proteine wurden mit Quecksilbersulfat ausgefällt. Die Trehalosebestimmungen mit Ausnahme einer Probe, 2b, wurden in den Proteincentrifugaten ausgeführt. In Probe 2b wurde Trehalose nach Fällung des HMPs bestimmt.

Zum ersten Versuche gehören die Proben 1–3. Probe 1 zeigt den Gehalt an Trehalose in der Acetondauerhefe, die zu diesem Versuche benutzt wurde. In den Proben 2 und 3 wurde die Neubildung von Trehalose ohne und in Gegenwart von $2 \times 10^{-3}M$ Natriumfluorid verglichen. Ohne Fluorid fand eine bedeutende Trehalosesynthese statt. Nach 5 Stunden wurden aus 100 mg zugesetzter Glucose 35,7 mg in Trehalose umgewandelt. Das Ausfällen des HMPs veränderte den Trehalosewert nur wenig (2a und 2b). In Gegenwart von Fluorid waren sowohl Gärung als auch Trehalosebildung vollständig gehemmt.

Im anderen Versuche wurden der Umsatz der Trehalose und die Konzentration anorganischen Phosphats während der Glucosegärung verfolgt. Proben wurden während 23 Stunden herausgenommen und analysiert. Der Gehalt an Trehalose in der für diesen Versuch verwendeten Acetonhefe geht aus den Proben 4 und 5 hervor. Probe 14 wurde zu gleicher Zeit als Probe 9 herausgenommen. Die Probemenge war jedoch hier 5mal grösser. Wie aus Tab. 2 hervorgeht, wurde die Trehalose nur aus der Zunahme des Reduktionswertes bestimmt. In denselben Proben, in denen die Trehalosebestimmungen ausgeführt wurden, wurde auch organisches Phosphat bestimmt. Dieses Esterphosphat wurde als HMP berechnet. In einem Teil der Gärproben wurde mit 5%iger Trichloressigsäure enteiweisst und in den Filtraten wurde Gesamtphosphat, organisches und anorganisches, bestimmt. Der Unterschied

zwischen dem organischen Phosphat in den Trichloressigsäurefiltraten und in den Quecksilberfiltraten wurde annähernd als die Konzentration von HDP berechnet. Verestertes Phosphat und damit äquivalente Glucose werden in Tab. 3 dargestellt.

In Fig. 3 sind die Werte vergorener Glucose, neugebildeter Trehalose, phosphorylierter Hexose und direkt reduzierender Substanz graphisch wiedergegeben. Aus Fig. 3 geht es hervor, dass die Konzentration des phosphorylierten Zuckers schon nach 15 Min. ein Maximum erreichte. Dabei waren noch etwa 25% des Phosphates unverestert. In dieser Hinsicht unterscheidet sich die Gärung der Dauerhefe von der des Mazerationssaftes, bei der, wie von NILSSON (31) hervorgehoben worden ist, kein anorganisches Phosphat vorhanden ist, wenn die Phosphorylierung ihr Maximum erreicht hat. Während der drei ersten Gärstunden blieb die Esterkonzentration beinahe unverändert. Während dieser Zeit war auch die Gärgeschwindigkeit am grössten. Zwischen der dritten und vierten Stunde sank die Gärgeschwindigkeit und die Konzentration veresterten Zuckers ab. Nach 6 Stunden war die Gärung hauptsächlich beendet. Die Esterkonzentration hatte da ein Minimum erreicht und blieb dann bis zum Ende des Versuches beinahe unverändert. Von Interesse ist zu beobachten, dass die Abnahme des phosphorylierten Zuckers nach 3 Stunden überwiegend auf HDP fiel. Vor 3 Stunden machte HDP 60% des Esterphosphates aus, nach 6 Stunden nur 40%.

Die Bildung der Trehalose scheint in einem gewissen Verhältnis zur Konzentration veresterten Zuckers zu stehen. Solange die Esterkonzentration konstant war, blieb auch die Synthesegeschwindigkeit einigermaßen konstant. Als die Esterkonzentration nach 3 Stunden zu sinken anfangte, nahm die Geschwindigkeit der Trehalosebildung beträchtlich ab, und als sie nach etwa 6 Stunden ein Minimum erreicht hatte, hörte die Synthese auf. Es trat eine langsame Hydrolyse des Disaccharides auf, die sich bei einer Bestimmung nach 8 Stunden in einer Verminderung der Trehalosekonzentration und in einer entsprechenden Zunahme direkt reduzierender Substanz zeigte.

Während der 3 ersten Gärstunden entsprach die neugebildete Trehalose 54–58% der vergorenen Glucose. Bemerkenswert ist, dass zwischen der vierten und sechsten Stunde, als den Reduktionswerten nach keine freie Glucose in den Versuchsmischungen mehr vorhanden war, 1,9 mg Trehalose neugebildet werden konnte. Dies könnte möglicherweise andeuten, dass das Disaccharid aus zwei phosphorylierten Hexosen aufgebaut wurde.

MYRBÄCK und ÖRTENBLAD (32) haben in der Presshefe eine Trehalase nachgewiesen, die Trehalose zu Glucose spaltet. Wahrscheinlich war es dieses Enzym, das am Ende der Glucosegärung das Disaccharid hydrolysierte. Man fragt sich aber, warum nur ein geringer Teil der aus Trehalose zurückgebildeten Glucose vergären konnte. Möglich ist, dass die Konzentration des ATPs, das für die Wirkung der Hexokinase notwendig ist, in der Zeit, in der Glucose wiedergebildet wurde, zu niedrig war, um die Phosphorylierung wieder in Gang zu bringen.

Zwischen der vierten und sechsten Stunde blieb die Konzentration der direkt reduzierenden Substanz unverändert. Nichtsdestoweniger nahm vergorene Glucose um 6,7 mg und Trehalose um 1,9 mg zu. Teilweise fanden diese Zunahmen auf Kosten der Phosphorsäureester statt, die um 3,4 mg abnahmen, der Rest aber muss von den Kohlehydraten der Hefe herrühren. TREVELYAN (9) fand ebenfalls, dass eine kurzzeitige, fortgesetzte Gärung auf Kosten des Hefeglykogens am Ende der Glucosegärung mit frischer Bäckerhefe entstand. Zwischen 6 und 23 Stunden machte die Zunahme vergorener Glucose und direkt reduzierender Substanz 43,9 mg aus. Wäh-

rend dieser Zeit war die Abnahme der Trehalose 41,8 mg und die der veresterten Glucose 1,5 mg. Hier war die Übereinstimmung sehr gut. Eine fortgesetzte Abnahme des Hefeglykogens scheint deswegen nicht stattgefunden zu haben.

Die letzte Kolumne der Tab. 3 stellt ein Zusammenzählen der analysierten Gärprodukte dar. Wenn der direkte Reduktionswert der Phosphorsäureester etwa 75 % des Glucosewertes beträgt, kann der direkte Reduktionswert der freien Glucose berechnet werden. Die berechneten Werte der Gärprodukte sind, besonders während der ersten Gärstunden, bedeutend niedriger als die Menge der zugesetzten Glucose. Nach 15 Min. ist das Defizit beispielsweise 20 %, nach 3 Stunden etwa 12 % und nach 8 Stunden 5 %. Das Defizit würde noch etwas höher werden, wenn die aus der Hefe herausgelösten Phosphorsäureester mitgezählt worden wären. Die Ergebnisse deuten darauf hin, dass sich während der Gärung Substanzen gebildet hatten, die nicht von den ausgeführten Analysen erfasst worden waren. TREVELYAN (9) fand, dass bei der anaeroben Gärung der Glucose mit frischer Bäckerhefe sich nicht unbedeutende Mengen Glyzerin bildeten. Möglich ist, dass dies auch hier der Fall war. Die Abnahme des Defizits am Ende der Gärung lässt sich dadurch erklären, dass ein Teil des Hefeglykogens abgebaut wurde.

b) Mit gewaschener Acetonhefe als Fermentsystem

Aufbau und Abbau der Trehalose vor und nach Fällung des HMPs bestimmt

Mit gewaschener Acetonhefe wurde der Umsatz der Trehalose bei Vergärung von Glucose in 0,05 M Phosphat in zwei Versuchen untersucht (Tab. 4 und 5). In dem zuerst ausgeführten wurde die Synthese der Trehalose nur bis zum Verschwinden der freien Glucose in den Gärproben verfolgt. An 4 verschiedenen Zeitpunkten wurden Doppelproben ausgenommen, in welchen die Trehalose in den Proteinzentrifugaten nur aus der Zunahme des Reduktionswertes bestimmt wurden. In einer Probe, in grösserem Masstabe ausgeführt, wurde Trehalose sowohl vor als auch nach Fällung des HMPs bestimmt. Im Esterfiltrat konnte die Bestimmung auch aus der optischen Aktivität ausgeführt werden. In allen Proben wurde freie Glucose mit Presshefe beseitigt und die Proben danach mit Quecksilbersulfat enteiweisst.

Probe 1 und 2 in Tab. 4 zeigen den Gehalt an Trehalose in dem Fermentsystem, das aus gewaschener Hefe und Kochsaft bestand. Um zu finden, ob die Trehalosekonzentration der Gärproben während der Beseitigung der freien Glucose mit Presshefe verändert wurde, wurden zwei Proben nach null Min. herausgenommen und wie die übrigen behandelt (3 und 4). Der Trehalosewert dieser Proben war 0,5 mg niedriger als der der Proben 1 und 2. Eine geringe Hydrolyse des Disaccharides wurde demnach von der Presshefe bewirkt. Nach MYRBÄCK (33) ist die Hefetrehalase wahrscheinlich in den äusseren Schichten der Hefemembran lokalisiert. Da die Presshefegärungen der folgenden Proben erheblich kürzere Zeiten als in den Proben 3 und 4 andauerten, schien es nicht nötig, eine Korrektur der Trehalasewirkung der Presshefe einzuführen.

Die Werte der Doppelproben in Tab. 4 stimmten durchwegs gut überein. Die Trehalosebildung zeigte in diesem Versuche mit gewaschener Hefe und Kochsaft fast genau denselben Verlauf wie im Versuche mit Acetondauerhefe (Tab. 2). Die neugebildete Trehalosemenge war jedoch etwas kleiner. Nach 65 Min. machte die neugebildete Trehalose etwa 40 % und nach 217 Min. 47 % der vergorenen Glucose aus.

Tab. 4. Synthese der Trehalose mit gewaschener Acetonhefe.

entwickelte Kohlensäure und Reduktionswerte als mg Glucose und organisches Phosphat als mg P berechnet.

Nr.	Gärzeit, Min.	Mit gewaschener Hefe vergorene Glucose	Mit frisch. Bäckerhefe vergorene Glucose	Reduzierende Substanzen		Diff.	Hydrolysegrad der Trehalose in %	Nach Korrr. unvollst. Hydrolyse	Neugebildete Trehalose in % vergor. Glucose	Organ. Phosphat in HgSO ₄ -filtr.
				vor Hydrolyse	nach Hydrolyse					
1				0,6	2,4	1,8	93,0	1,9		
2				0,7	2,5	1,8	»	1,9		
3	0	0	96,0	0,7	1,9	1,2	86,5	1,4		0,69
4	0	0	96,0	0,9	2,1	1,2	»	1,4		
5	65	22,5	52,5	5,2	14,6	9,4	85,7	11,0	40,5	1,88
6	65	22,5	52,5	5,1	14,3	9,2	»	10,7	39,1	
7	160	45,0	18,5	5,6	24,6	19,0	»	22,2	45,1	1,82
8	160	45,0	18,5	5,4	24,4	19,0	90,2	21,1	42,7	
9	217	56,2	2,5	4,0	28,2	24,2	85,7	28,2	46,8	1,89
10	217	56,2	2,5	4,1	29,5	25,4	90,2	28,2	46,8	
11	310	67,5	0	3,4	29,1	25,7	86,5	29,7	41,2	1,82
12	310	67,5	0	3,7	30,8	27,1	90,2	30,0	41,7	
13	255	61,5		2,3	25,4	23,1	73,0	31,6	48,3	1,17
14				0,4	21,2	20,8	67,2	31,0	47,3	

Nach Ausfällen des HMPs.

Tab. 5. Umsatz der Trehalose mit gewaschener Acetonhefe.

Alle Werte als mg Glucose berechnet.

Nr.	Gärzeit, Min.	Mit gewaschener Hefe vergorene Glucose	Mit frisch. Bäckerhefe vergorene Glucose	Trehalose in Proteinfiltr. aus		Trehalose in Esterfiltr. aus		Neugebildete Trehalose in % vergor. Glucose	Diff. der Red.-Werte in %
				Zunahme des Red.-Wertes	optischer Aktivität	Zunahme des Red.-Wertes	optischer Aktivität		
1				5,7	5,4	5,6	5,2		- 1,8
2				0	0	0	0		
3	30	14,2	69,8	8,9	9,8	11,7	11,3	43,0	+ 23,9
4	30	12,6	69,2			15,3	14,3	77,0	
5	100	35,4	36,4	14,4	15,8	14,5	13,2	25,1	+ 0,7
6	100	31,9	41,7			13,2	13,5	23,8	
7	165	49,5	13,7	19,0	20,9	19,6	19,6	28,3	+ 3,1
8	200	56,3	—	19,9	21,1	19,0	18,5	23,8	- 4,7
9	240	65,5	0			21,7	20,8	24,6	
10	300	73,3	0	19,0		21,0	18,3	21,0	+ 9,5
11	420	78,4	2,1	12,3	11,2	10,5	9,9		- 17,1
12	600	92,2	0,6	2,0		2,4	1,3		+ 16,7
13	1 440	100,6	1,6	1,7	*	1,5	*		- 13,3
14	100	25,0		6,8	6,6	6,4	5,2		
15	165	33,2		5,6		4,9	3,7		

* Negative optische Aktivität.

Die Trehalosekonzentration der Probe 13 veränderte sich nur unbedeutend durch die Entfernung des HMPs. Die Trehalosewerte aus der Reduktionszunahme und aus der optischen Aktivität stimmten gut überein.

Die Konzentrationen freien und veresterten Phosphates wurden in den Versuchen mit gewaschener Hefe und Kochsaft erheblich grösser als in den Versuchen mit Acetondauerhefe, was teils davon, dass die Hefemenge 50 % grösser war, teils davon, dass Hefekochsaft und HDP-Lösung zugesetzt werden mussten, abhing. Dadurch wurde auch die Konzentration des organischen Phosphats in den Quecksilberfiltraten erheblich grösser, was die Trehalosebestimmungen unsicherer machten. Zwar veränderte sich der Trehalosewert der Probe 13 durch die Fällung des HMPs nur um etwa 3 %, die Konzentration des organischen Phosphats dieser Probe war aber bedeutend niedriger als die der übrigen.

In den Proben 5–12 war organisches Phosphat mit 10–11 mg Glucose äquivalent (Tab. 4). Da freie Glucose aus den Gärproben entfernt worden war, konnte die Konzentration der reduzierenden Ester, d. h. G-6-P und F-6-P, berechnet werden. Durch den Unterschied zwischen organischem und reduzierendem Esterphosphat wurde die Konzentration der nicht reduzierenden Ester bestimmt. Nach 160 Min. war sie als Glucose berechnet 3,1 mg und nach 310 Min. 6,0 mg. Diese Ester würden also $\frac{1}{3}$ bzw. $\frac{1}{2}$ des HMPs ausmachen. COLOWICK und SUTHERLAND (34) fanden, dass beim Gleichgewicht eines Estergemisches von G-1-P und G-6-P, mit gereinigter Phosphoglucomutase aus Muskel, 5,5 % des Gemisches bei 30° aus G-1-P bestand. In Mazerationssaft aus *Saccharomyces cerevisiae* fand Kosterlitz (35), dass 83 % zugesetzten G-1-Ps in G-6-P umgewandelt wurde. Im Verhältnis zu diesen Ergebnissen schien die Konzentration der nicht reduzierenden Ester zu gross zu sein, um allein aus G-1-P bestehen zu können.

Das Vorkommen von THP, dass von ROBISON und MORGAN (14) eben aus Acetondauerhefegärungen isoliert worden ist, schien nach dem Obengesagten als sehr wahrscheinlich. Da dieser Ester, der nach den genannten Verfassern durch Säurehydrolyse in G-6-P und Glucose hydrolysiert wird, beinahe mit derselben Geschwindigkeit wie Trehalose gespalten wird und wie Trehalose auch hohe optische Aktivität zeigt, $[\alpha]_D = +155^\circ$, so werden die Trehalosebestimmungen dieser Untersuchung auch THP umfassen.

Das neutrale Bariumsalz des THPs wird nach ROBISON und MORGAN (14) mit 75 % Alkohol bei pH 8,4 zum grössten Teile ausgefällt. Der Ester ist jedoch erheblich löslicher als G-6-P und F-6-P. THP aus trehalosehaltigen Lösungen zu entfernen, wird dadurch erschwert, dass Trehalose bei einer Alkoholkonzentration von etwa 80 % herauszukristallisieren beginnt.

Im Vorgehenden ist in einigen Versuchen gezeigt worden, dass nach dem Ausfällen des HMPs der Trehalosewert sich nur unbedeutend veränderte. Dies deutete nicht darauf hin, dass THP, sofern es vorkam, in dieser Weise entfernt werden konnte. Diese Bestimmungen wurden jedoch nur am Ende der Glucosegärungen ausgeführt. Die Möglichkeit bestand, dass grössere Differenzen der Trehalosewerte entstanden, wenn die Bestimmungen während des früheren Teiles der Gärung ausgeführt worden wären.

Ein zweiter Versuch mit gewaschener Acetonhefe wurde ausgeführt. Hier wurde die Trehalosekonzentration vor und nach dem Ausfällen des HMPs bestimmt, um

¹ Nach ROBISON und MORGAN (14) ist $[\alpha]_{5461} = +185^\circ$. Daraus lässt sich nach COLOWICK und SUTHERLAND (34) $[\alpha]_D = 0,84 \times +185^\circ = +155^\circ$ berechnen.

herauszufinden, ob ein Unterschied entstand, der auf ein teilweises Ausfällen des THPs deutete.

Während 24 Stunden wurden die Gärproben sukzessiv herausgenommen, freie Glucose mit Presshefe vergoren und die Proben mit Quecksilbersulfat enteieisist. Die Trehalosekonzentration wurde sowohl aus der Zunahme des Reduktionswertes als auch aus der optischen Aktivität bestimmt (Tab. 5). Proben 1 und 2 zeigen den Gehalt an Trehalose im Kochsaft und in der gewaschenen Hefe. Wie ersichtlich ist, war die letztere durch das Waschen der Hefe vollkommen von Trehalose befreit. Proben 3–13 zeigen den Umsatz der Trehalose bei der Vergärung von Glucose, Proben 14 und 15 die Synthese der Trehalose bei endogener Gärung der Acetonhefe.

Die Neubildung der Trehalose war in diesem Versuche erheblich niedriger als in den in Tab. 2 und 4 dargestellten. Das Maximum der Trehalosebildung wurde nach etwa 240 Min. erreicht, dabei hatte sich nur eine Trehalosemenge, mit 24,6% vergorener Glucose äquivalent, gebildet, während in Tab. 2 und 4 dieselben Werte 56,0 und 41,5% waren. Die Konzentration der Trehalose nahm hier nicht mit der Zeit proportional zu, sondern die Zunahme am Anfang der Gärung war auffallend gross. Der eine nach 30 Min. bestimmte Wert war sogar grösser als die beiden nach 100 Min. bestimmten Werte. Dies deutete darauf hin, dass die Voraussetzungen für den Aufbau der Trehalose während dieses Zeitabschnittes der Gärung am grössten waren. Dass dies Verhältnis nicht auch im vorigen Versuche nachgewiesen werden konnte, könnte davon abhängen, dass die erste Trehalosebestimmung zuerst nach 65 Min. ausgeführt wurde, wo die Synthesegeschwindigkeit schon gesunken war. Es war auch möglich, dass dieser Trehalosewert durch die Einwirkung von HMP etwas zu niedrig war, was nicht unwahrscheinlich ist, wenn man die grosse Zunahme der Trehalose nach Ausfällen des HMPs in der 30-Minuten-Probe der Tab. 5. beobachtet.

Werden die aus der Zunahme der Reduktion bestimmten Trehalosewerte verglichen, kann keine ausgesprochene Tendenz der Werte nachgewiesen werden. In den meisten Proben sind doch die Werte der Esterfiltrate am höchsten, was nicht darauf deutet, dass THP zusammen mit HMP ausgefällt wurde, sondern im Gegenteil, dass die Trehalosebestimmungen durch die Einwirkung von Fructoseestern zu niedrig werden konnten.

Werden die Trehalosewerte aus der optischen Aktivität verglichen, sind sie, mit Ausnahme von Probe 3, niedriger nach dem Ausfällen der Ester als vorher. Da die spezifische Drehung der ausgefällten Phosphorsäureester, d.h. G-6-P, F-6-P und G-1-P, positiv ist, sinkt die optische Aktivität, wenn sie entfernt werden. Die Bestimmungen der optischen Aktivität der Proteinfiltrate wurden durch Gegenwart von kolloidalem Quecksilbersulfid unzuverlässig, weshalb die Änderung der optischen Aktivität nach dem Ausfällen der Phosphorsäureester nicht genau bestimmt werden konnte. Enthielt die Hexosemonophosphorsäurefällung THP, musste jedoch die Konzentration davon, nach dem oben Gesagten, niedrig gewesen sein.

Die Übereinstimmung der Trehalosewerte, die nach den beiden Analysemethoden in den Esterfiltraten bestimmt wurden, war meistens gut. Die zuverlässigsten Werte waren die aus der Zunahme des Reduktionswertes bestimmten. Die Fehler bei den Zuckerbestimmungen betrugen etwa $\pm 3\%$. Bei der Bestimmung der optischen Aktivität konnte, wenn der abgelesene Wert niedrig war, der Ablesungsfehler bis auf 15–20% ansteigen.

In Tab. 6a sind die Phosphorsäurebestimmungen in den Quecksilbersulfatfiltraten und in den Ester- und Trehalosefraktionen dargestellt. Die Summe des organischen

Phosphats der Fraktionen stimmte gut mit dem Esterphosphat der Proteinfiltrate überein. Zum Vergleiche mit den Phosphorsäurewerten sind die direkten Reduktionswerte auch in diese Tabelle eingeführt. Die Summe der Reduktionswerte in den Fraktionen war in einigen Proben etwas niedriger als in den Proteinfiltraten. Der Anlass dazu ist jedoch nicht klar.

Aus Kochsaft und aus Hefeextrakt wurde nur eine geringe Menge von Esterphosphat ausgefällt. Dieses organische Phosphat zeigte keine Reduktion. Die Konzentration der ausgefallenen Phosphorsäureester der Glucosegärproben machte zwischen 62 und 77 % der Gesamtkonzentration des Esterphosphates der Proteinfiltrate aus. Bis auf die fünfte Gärstunde war ihre Konzentration ziemlich konstant. Nach 7 Stunden war sie bis auf 55 % gesunken. Nachdem zugesetzte freie Glucose verbraucht worden war, nahm die Konzentration des Esterphosphates der Esterfraktion um etwa 45 % ab, während die Konzentration des Esterphosphates der Trehalosefraktion beinahe unverändert blieb.

In den Ester- und Trehalosefraktionen wurde das Phosphat bestimmt, das nach 10 und 180 Min. in 1 *N* Schwefelsäure bei 100° abgespalten wurde. Nach 10 Min. ist G-1-P vollständig und F-6-P nur bis auf 8 % hydrolysiert (36). Das 10-Minuten Hydrolysephosphat gibt deswegen einen annähernden Wert der Konzentration von G-1-P an. Nach 180 Min. sind nach LEPAGE (25) 74 % des F-6-Ps, 10,5 % des G-6-Ps¹ und nach ROBISON und MORGAN (14) 4 % des THPs hydrolysiert. Das nach 180 Min. nicht hydrolysierte Phosphat sollte demnach hauptsächlich aus G-6-P und THP bestehen.

In der Esterfraktion wurde die Konzentration des THPs aus dem Unterschied zwischen nicht reduzierendem und leicht hydrolysierbarem Esterphosphat bestimmt (Tab. 6b). THP ist in der Tabelle als 2 Äquivalente Glucose berechnet. Die Konzentration des Esters war in dieser Fraktion sehr niedrig und blieb, solange die Gärung andauerte, konstant. Die Werte des leicht hydrolysierbaren Phosphates sind im Verhältnis zu der Gesamtmenge der ausgefallenen Ester auffallend gross. Die THP-Werte müssen deswegen bis auf weiteres mit Reservation angenommen werden, solange die genaue Konzentration des G-1-Ps nicht bekannt ist. Es war allerdings möglich, dass das HMP mit kleinen Mengen von HDP vermischt war. Dieser Ester wirkt dann sowohl auf die Konzentration des leicht hydrolysierbaren als auch auf die des nicht reduzierenden Esterphosphates ein. Nach HERS *et al.* (36) ist der Ester nach 10 Min. bei 100° bis auf 35 % hydrolysiert. Sein Reduktionswert ist mit 59 % des Glucosewertes äquivalent.

Das Esterphosphat der Trehalosefraktionen war während der ganzen Versuchszeit ziemlich konstant. Ein sicherer Reduktionswert des organischen Phosphats dieser Fraktion konnte nicht erhalten werden, da die reduzierenden Substanzen unbekannt waren. Das organische Phosphat des Kochsaftes und Hefeextraktes machte 0,37 mg und das der Gärproben zwischen 0,40 und 0,50 mg aus. Leicht hydrolysierbares Esterphosphat war aus diesen Fraktionen vollkommen entfernt. Die Werte des nach 180 Min. hydrolysierten Esterphosphates zeigen, dass es ausschliesslich aus schwerhydrolysierbarem Phosphat bestand. Da das G-6-P aus dieser Fraktion entfernt worden war kann es zum Teil aus THP bestanden haben. Nach dem oben Gesagten, kann vorläufig die Konzentration des Esters weder in den Gärproben noch im Hefekochsaft bestimmt werden. Aus den Werten des THPs der Esterfraktionen und aus der Menge organisches Phosphats der Trehalosefraktionen

¹ Diese Werte sind in 1 *N* HCl, 100°, erhalten.

Tab. 6a. Fraktionierung der Esterphosphate.

Reduktionswerte als mg Glucose und organisches Phosphat als mg P berechnet.

Nr.	Gärzeit, Min.	Proteinfiltr.		Esterfraktionen				Trehalosefraktionen		
		Org. Phosphat	Direkte Red.- Werte	Org. Phosphat	Direkte Red.- Werte	Nach 10 Min. hydr. Phosphat	Nach 180 Min. hydr. Phosphat	Org. Phosphat	Direkte Red.- Werte	Nach 180 Min. hydr. Phosphat
1		0,28	0,1	0,03	0			0,28	0,1	
2		0,15	1,7	0,05	0			0,09	0,7	
3	30	1,43	3,4	1,03	2,7			0,50	0,4	
4	30			1,24	3,9	0,26	0,60	0,42	0,7	0,03
5	100	1,87	5,4	1,28	3,8			0,49	1,1	
6	100			1,51	4,4	0,43	0,84	0,44	0,8	0,03
7	165	2,08	6,7	1,30	3,8			0,68	2,7	
8	200	1,19	5,1	0,80	2,1	0,21	0,44	0,40	1,8	
10	300	1,99	5,2	1,53	3,4			0,47	0,9	0,03
11	420	1,33	3,6	0,84	2,0	0,30	0,51	0,40	0,5	0,02
12	600	1,36	5,8	0,87	2,2	0,33	0,61	0,47	1,0	0,04
13	1 440	1,36	6,2	0,85	2,2	0,32	0,54	0,53	1,8	0,02
14	100	0,70	0,9	0,30	0,4			0,41	0,2	
15	165	0,72	0,7	0,28	0,2			0,41	0,2	

Tab. 6b. Berechnete Konzentration des THPs in den Esterfraktionen.

Alle Werte in mg Glucose ausgedrückt. THP als zwei Äquivalente Glucose berechnet.

Nr.	Org. Phosphat	Esterfraktionen		
		Nicht redu- zierende Ester	Leicht hydro- lysierbare Ester	THP
1	0,17	0,17		
2	0,29	0,29		
3	6,0	2,4		
4	7,2	2,0	1,5	1,0
5	7,4	2,3		
6	8,8	2,9	2,5	0,8
7	7,6	2,5		
8	4,6	1,8	1,2	1,2
10	8,9	4,4		
11	4,9	2,2	1,7	1,0
12	5,1	2,2	1,9	0,6
13	4,9	2,0	1,9	0,2
14	1,7	1,2		
15	1,6	1,3		

Tab. 7. Synthese der Trehalose bei verschiedenen Glucosekonzentrationen.

Alle Werte als mg Glucose ausgedrückt.

Nr.	Gärzeit, Min.	Mit gewaschener Hefe vergorene Glucose	Mit frisch. Bäckerhefe vergorene Glucose	Reduzierende Substanzen		Diff.	Hydrolysegrad der Trehalose in %	Nach Korr. unvollst. Hydrolyse	Neugebildete Trehalose in % vergorener Glucose
				vor Hydrolyse	nach Hydrolyse				
1				0,7	4,3	3,6	78,3	4,6	
2				0,6	4,3	3,7	»	4,7	
3	60	28,4	4,5	1,7	17,2	15,5	79,6	19,5	52,1
4	160	56,3	3,0	2,0	30,1	28,1	»	35,3	54,4
5	370	83,7	11,9	1,9	39,3	37,4	»	47,0	50,5

kann jedoch geschlossen werden, dass die Gesamtkonzentration des THPs während der ganzen Versuchszeit niedrig und ziemlich konstant war, was darauf deutet, dass es sich um ein Zwischenprodukt handelt.

Es wurde im Vorgehenden gezeigt, dass die Glucosegärung mit Acetondauerhefe aufhörte, wenn 50–60 mg zugesetzter Glucose vergoren waren. Dies war nicht der Fall, wenn gewaschene Dauerhefe und Kochsaft gebraucht wurden. Wenn etwa 50 mg Glucose vergoren waren, nahm freilich die Gärgeschwindigkeit schnell ab, die Gärung setzte jedoch fort, bis eine Kohlensäuremenge, die mit etwa 100 mg Glucose äquivalent war, gebildet wurde. Die Anhäufung der Glucose, die sich durch die Hydrolyse der Trehalose in Versuchen mit Acetondauerhefe bildete, entstand hier nicht, sondern die Glucose und ein Teil der Phosphorsäureester wurden vergoren.

MEYERHOF (37, 38) hat gezeigt, dass ein Ferment, das ATP hydrolysiert, leicht beim Trocknen der Hefe geschädigt wird. Wenn die Aktivität dieses Enzymes durch das Waschen und Trocknen der Acetonhefe herabgesetzt wurde, kann die Folge hiervon gewesen sein, dass die Konzentration des ATPs während der Gärung mit gewaschener Hefe grösser als mit Acetondauerhefe war. Es ist auch möglich, dass das Fermentsystem dadurch, dass die Hefekonzentration grösser war und dass Hefekochsaft zugesetzt werden musste, eine grössere Konzentration von ATP besass, wodurch neugebildete Glucose leichter wiederphosphoryliert werden konnte.

Wie in Tab. 5 gezeigt wurde, war die Neubildung der Trehalose am Anfang dieses Gärungsversuches auffallend gross. Um feststellen zu können, ob das Hefeglykogen hierbei beteiligt war, wurde die Neubildung von Trehalose in zwei Gärproben, zu welchen keine Glucose zugesetzt wurde, nach 100 und 165 Min. bestimmt. Die Zusammensetzung der Proben war sonst wie die der übrigen und sie wurden auch nach der Gärung wie diese behandelt. Nach 100 Min. war die Trehalosekonzentration, aus der Zunahme des Reduktionswertes berechnet, um 0,8 mg höher als die der Nullprobe. Nach 165 Min. war der Wert um 0,7 mg niedriger. Die Neubildung der Trehalose aus Glykogen allein war demnach unbedeutend. Damit ist jedoch nicht bewiesen, dass das Glykogen nicht eine gewisse Rolle bei dem Aufbau der Trehalose bei der Glucosegärung spielen kann. Es scheint jedoch am wahrscheinlichsten zu sein, dass die Phosphorolyse des Glykogens durch die Glucosephosphorylierung zurückgedrängt wird und erst in Gang kommen kann, wenn die Glucose verbraucht ist.

Tab. 8. Vergleich der Trehalosebildung in 0,05 und 0,15 M Phosphat.

Alle Werte als mg Glucose ausgedrückt.

Nr.	Gärzeit, Min.	Mit gewasch. Hefe vergorene Glucose	Mit frisch. Bäckerhefe vergorene Glucose	Trehalose aus optischer Aktivität		In Esterfiltrate reduzierende Substanzen		Diff.	Hydrolysegrad der Trehalose in %	Nach Korr. unvollst. Hydrolyse
				in Prot.-filtr.	in Ester-filtr.	vor Hydrolyse	nach Hydrolyse			
1	190	58,2	9,3	30,7	30,2	1,0	26,4	25,4	79,9	31,8
2	»	58,2	9,3	30,0	31,2	1,2	28,2	27,0	»	33,8
3	110	56,0	0	30,3	29,0	0,9	24,8	23,9	79,0	30,2
4	»	56,0	0		31,2	1,0	27,8	26,8	»	33,9

Tab. 9. Synthese der Trehalose in 0,10 M Phosphat.

Alle Werte wie in der vorgehenden Tabelle ausgedrückt.

Nr.	Gärzeit, Min.	Mit gewasch. Hefe vergorene Glucose	Mit frisch. Bäckerhefe vergorene Glucose	Trehalose in Proteinfiltr. aus Zunahme der Reduktionswerte	Trehalose in Esterfiltraten aus		Neugebildete Trehalose in % vergorener Glucose
					Zunahme der Red.werte	optischer Aktivitäten	
1	30	8,6	69,0	7,5	6,4	6,6	9,3
2	100	39,0	22,4	15,9	16,4	15,8	27,7
3	165	62,2	1,2	18,7	19,6	18,7	22,5

Die Konzentration des organischen Phosphates bei der endogenen Gärung wird in Tab. 6a gezeigt. Das Phosphat der Esterfraktionen betrug nur 20–25 % des gefällten Esterphosphates der Glucosegärproben. In den Trehalosefraktionen aber war die Konzentration des Esterphosphates nur unbedeutend niedriger als die der meisten Glucosegärproben.

Trehalosebildung bei verschiedenen Glucosekonzentrationen

Die Neubildung der Trehalose wurde bei drei verschiedenen Glucosekonzentrationen, 50, 100 und 150 mg pro Gärprobe, untersucht (Tab. 7). Die Gärungen wurden unterbrochen, wenn 56 % der zugesetzten Glucose vergoren waren. Freie Glucose wurde mit Presshefe entfernt und die Proben mit Bariumhydroxyd und Zinksulfat nach Somogyi enteiweisst. Da die Konzentration des organischen Phosphates in diesen Proteinfiltraten niedriger war als in den Filtraten, wo mit Quecksilbersulfat enteiweisst wurde, so wurden die Einwirkungen der Phosphorsäureester auf die Trehalosebestimmungen reduziert. Aus den Werten von Tab. 7 geht hervor, dass neugebildete Trehalose zu der zugesetzten Glucose proportional und mit etwa 50 %

Tab. 10. Fraktionierung der Esterphosphate.

Reduktionswerte als mg Glucose und organisches Phosphat als mg P ausgedrückt.

Nr.	Gärzeit, Min.	Proteinfiltrate		Esterfraktionen			Trehalosefraktionen	
		Org. Phosphat	Direkte Red.-Werte	Org. Phosphat	Direkte Red.-Werte	Nach 10 Min. hydr. Phosphat	Org. Phosphat	Direkte Red.-Werte
1	30	2,50	6,8	2,03	5,3	0,41	0,41	0,9
2	100	3,71	8,4	3,09	6,4	0,85	0,48	0,9
3	165	3,09	6,2	2,67	4,1	0,85	0,44	0,8

vergorener Glucose äquivalent war. Es ist aus der Tabelle ersichtlich, dass, je grösser die Glucosekonzentration war, desto langsamer wurde die Gärgeschwindigkeit und damit auch die Trehalosesynthese. Dies Verhältnis zeigt wieder die Abhängigkeit der Trehalosebildung von den bei der Gärung gebildeten Phosphorsäureestern.

4. Aufbau der Trehalose in Gegenwart von 0,10 und 0,15 M Phosphat

Bei Phosphatkonzentrationen höher als 0,05 M ist vorher gezeigt worden, dass das Phosphat am Anfang der Gärung die Gärgeschwindigkeit hemmte. Wenn die Hemmung überwunden worden war, nahm die Gärgeschwindigkeit schnell zu und wurde beträchtlich höher als in Proben mit 0,05 M Phosphat (Fig. 1 und 2).

In zwei Versuchen wurde die Einwirkung von 0,10 und 0,15 M Phosphat auf die Synthese der Trehalose untersucht. In dem einen Versuch wurde die maximale Trehalosebildung bei 0,05 und 0,15 M Phosphat verglichen (Tab. 8). In dem anderen Versuch, wo die Phosphatkonzentration 0,10 M war, wurde die Trehalose nach 30, 100 und 165 Min. bestimmt, um herauszufinden, ob die Hemmung der Gärgeschwindigkeit auch auf die Trehalosebildung einwirkte (Tab. 9 und 10).

In dem erstgenannten Versuche wurden die Gärproben herausgenommen, wenn die freie Glucose verbraucht worden war, und wie die Proben in Tab. 4 behandelt. In den Proteinfiltraten wurde die Trehalose nur aus der optischen Aktivität bestimmt. HMP wurde in üblicher Weise ausgefällt und in den Esterfiltraten die Konzentration der Trehalose, sowohl aus der Zunahme des Reduktionswertes als auch aus der optischen Aktivität, festgestellt. Proben 1 und 2 in Tab. 7 zeigen den Gehalt an Trehalose des Fermentsystemes. Proben 1 und 2 in Tab. 8 geben die Neubildung der Trehalose bei 0,05 M Phosphat und Proben 3 und 4 die bei 0,15 M Phosphat an. Aus den Werten geht es hervor, dass die maximale Trehalosebildung bei den beiden untersuchten Phosphatkonzentrationen dieselbe war. Die Synthese verlief aber bei der höheren Phosphatkonzentration wesentlich schneller. Aller Wahrscheinlichkeit nach beruht dies darauf, dass die Konzentration der veresterten Glucose in diesem Fall höher war. Bemerkenswert ist, dass die Trehalosebestimmungen aus der optischen Aktivität in Protein- und Esterfiltraten so gut übereinstimmen. Nur kleine Mengen von THP können deswegen zusammen mit HMP ausgefällt sein.

In Tab. 9 werden die Trehalosewerte in dem Versuche mit 0,10 M Phosphat dargestellt. Der Versuch wurde wie der in Tab. 5 ausgeführt. Dasselbe Ferment system wurde auch verwendet. Fig. 4 vergleicht vergorene Glucose und neugebil-

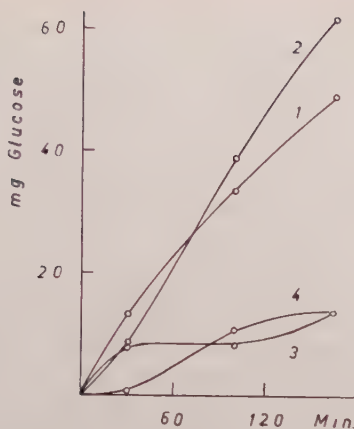


Fig. 4. Vergleich der Trehalosebildung in 0,05 *M* und 0,10 *M* Phosphat.

1. Vergorene Glucose, 3. neugebildete Trehalose in 0,05 *M* Phosphat.
2. Vergorene Glucose, 4. neugebildete Trehalose in 0,10 *M* Phosphat.

gebildete Trehalose bei 0,05 und 0,10 *M* Phosphat. Bei der niedrigeren Phosphatkonzentration wurde, wie vorher gezeigt, die Hauptmenge der Trehalose während der ersten 30 Min. gebildet. Die Konzentration nahm danach äusserst langsam zu. Bei der höheren Phosphatkonzentration war die Trehalosesynthese während der ersten Halbstunde der Gärung beinahe vollständig gehemmt. Danach nahm die Konzentration des Disaccharides mit relativ grosser Geschwindigkeit zu und hatte nach 100 Min. beinahe dieselbe Konzentration wie bei 0,05 *M* Phosphat erreicht. Nach 165 Min. waren auch die Trehalosewerte gleich gross, weshalb die maximale Trehalosebildung, wie im vorigen Versuche, innerhalb gewisser Grenzen von der Konzentration des Phosphates unabhängig zu sein scheint.

Aus Tab. 10 geht hervor, dass nach 30 Min. die Konzentration des organischen Phosphats beinahe doppelt so gross als in Gegenwart von 0,05 *M* Phosphat ist. Die Hemmung der Synthese wurde deswegen nicht dadurch verursacht, dass die Phosphorylierung der Glucose niedriger war. Die nach 30 Min. vergorene Glucose machte 60–70 % der bei 0,05 *M* Phosphat vergorenen aus. Die Gärung und damit auch die Bildung des ATPs wurden dann viel weniger gehemmt als die Synthese. Der Grund der Synthesehemmung war darum nicht leicht zu durchschauen. Es war aber möglich, dass die grössere Konzentration des anorganischen Phosphats am Anfang des Versuches die Trehalosebildung hemmte. Wenn THP bei der Trehalosebildung ein Zwischenprodukt ist, so wäre es eine plausible Erklärung, dass das anorganische Phosphat die Phosphatasespaltung dieses Esters hemmt.

In der Esterfraktion wurde das nach 10 und 180 Min. hydrolysierbare Phosphat bestimmt. Macht man hier einen Versuch, die Konzentration des THPs aus der Differenz zwischen nicht reduzierendem und leicht hydrolysierbarem Esterphosphat zu berechnen, werden Werte um 5 bis 10 mg THP erhalten. Aus den Trehalosebestimmungen ist jedoch ersichtlich, dass diese Werte nicht richtig sein können. Sie können nur dadurch erklärt werden, dass eine nicht unbedeutende Menge von HDP in diesen Proben vorkam, die mit den Proteinen nicht entfernt worden war, weil die Gesamtkonzentration des Esters erheblich grösser war als im Versuche mit 0,05 *M* Phosphat. In den Trehalosefraktionen war die Konzentration des organischen Phosphates genau dieselbe wie in den Trehalosefraktionen mit 0,05 *M* Phosphat.

5. Verhältnis zwischen zugesetzter Substratmenge und analysierten Umsatzprodukten der Acetonhefegärung

In der in Tab. 2 und 3 wiedergegebenen Untersuchung des Trehaloseumsatzes wurde gezeigt, dass nach 3 und 8 Stunden Gärung die Summe der analysierten Stoffwechselprodukte 88 bzw. 95% zugesetzter Glucose ausmachte. Wie erwähnt wurde, muss das Defizit etwas grösser gewesen sein, da die veresterte, aus der Hefe herausgelöste Hexose nicht bestimmt wurde.

In zwei gleichlaufenden Gärungsversuchen mit gewaschener Hefe und Kochsaft wurde dieser Vergleich zwischen zugesetzter Substratmenge und analysierten Gärprodukten wiederholt. Die veresterte Hexose im Wasserextrakt der Acetonhefe und im Kochsaft wurden hier bestimmt (Tab. 11, Probe 1 und 2). Das Fermentations-system war dasselbe wie in Tab. 7. Ein Teil der Gärproben wurde mit Quecksilbersulfat enteiweisst. Nachdem HMP in üblicher Weise entfernt worden war, wurde Trehalose in diesen Proben aus der Zunahme des Reduktionswertes bestimmt. Ein anderer Teil der Proben wurde mit 5%iger Trichloressigsäure enteiweisst. Nach ROBISON und MORGAN (14) wurde HDP aus diesen Proben mit Bariumacetat, Bariumhydroxyd und 10%igem Alkohol bei pH 8,4 und HMP nach LEPAGE (25) wie oben ausgefällt.

Tab. 11 stellt eine Übersicht der Konzentrationen freien und veresterten Phosphates der verschiedenen Fraktionen und die Konzentration neugebildeter Trehalose dar. Das Gesamtphosphat der Gärproben überstieg um 0,40 mg für Probe 3 und um 0,52 mg für Probe 4 die Summe zugesetzten, anorganischen und organischen, Phosphates. Der Grund für diesen Unterschied muss darin zu suchen sein, dass mehr Phosphat aus der Hefe bei den Gärungsversuchen als bei der Wasserextraktion ausgelöst wurde. In Tab. 3 wird gezeigt, dass auch während der Gärung mit Acetondauerhefe ein Austritt von Phosphat stattfand. Nach 23 Stunden Gärung war das Gesamtphosphat, aus zugesetztem anorganischem Phosphat und aus Phosphat des Hefeextraktes berechnet, um 0,40 mg niedriger als das Phosphat der Gärprobe. Es wird angenommen, dass dieser Phosphataustritt wenigstens teilweise von Phosphat-tasen verursacht wird, die in der Hefe gebundene Phosphorsäureverbindungen wie Phosphoproteide und Nucleoproteide, angreifen. Teilweise kann der Phosphataustritt auch durch Hydrolasen bewirkt worden sein, die organisches Phosphat abspalten.

Die Konzentration des organischen Phosphates des Proteinfiltrates war um 0,3 mg grösser als die Konzentration der Fraktionen. Wahrscheinlich bestand das vermisste organische Phosphat aus HMP, das durch das Auswaschen des Hexosemonophosphatniederschlages mit 75%igem Alkohol entfernt wurde, da die Waschflüssigkeit weggeworfen wurde. Wird das vermisste Phosphat als HMP berechnet, stimmen die Werte des organischen Phosphates der Ester- und Trehalosefraktionen mit den Werten in Tab. 6a ausgezeichnet überein.

Aus Tab. 11 ist ersichtlich, dass nach einer Gärzeit von 230 Min. 60% des organischen Phosphates aus HDP und 38% des Gesamtphosphates aus anorganischem Phosphat bestanden. HDP machte die Hauptmenge des organischen Phosphates des Hefeextraktes und des Kochsaftes aus, während die Konzentration des HMP unbedeutend war.

Die Gesamtkonzentration der analysierten Gärprodukte, als Glucose berechnet, betrug 100,6 mg in Probe 3 und 101,1 mg in Probe 4. Das vermisste Phosphat, als HMP berechnet, war mit etwa 2 mg Glucose äquivalent. Wenn diese Menge zu den

Tab. 11. Verteilung der Esterphosphate bei der Gärung mit gewaschener Hefe in 0,05 M Phosphat.

3,36 mg anorganisches Phosphat und 0,39 mg organisches Phosphat (HDP) wurden pro Gärprobe zugesetzt. Mit Acetonhefe waren in beiden Proben nach 230 Min., wenn die Gärung unterbrochen wurde, 56,2 mg Glucose vergoren. Mit frischer Bäckerhefe wurden weiter 2 mg Glucose beseitigt. Alle Werte der Tabelle in mg.

Nr.	Proteinfiltr.		HDP-Fractionen			HMP-Fractionen			Trehalose-Fractionen	
	Anorg. Phosph.	Org. Phosph.	Anorg. Phosph.	Org. Phosph.	Äquival. Glucose	Anorg. Phosph.	Org. Phosph.	Äquival. Glucose	Neugeb. Trehalose als äquiv. Glucose	Org. Phosph.
1	0,84	0,76	0,80	0,42	1,2		0,06	0,3		0,28
2	0,06	1,03	0,05	0,89	2,6		0,05	0,3		0,09
3	2,56	4,28	2,42	2,54	7,4	0,13	0,90	5,2	29,8	0,45
4	2,64	4,32	2,38	2,60	7,6	0,12	0,90	5,2	30,1	0,43

Obengenannten hinzugefügt wird, erhält man etwa 103 mg, was überraschend gut mit dem berechneten Werte, 105,5 mg, übereinstimmt. Die gute Übereinstimmung kann so gedeutet werden, dass das Phosphat, das während der Gärung aus der Hefe herausgelöst wurde, zum überwiegenden Teile aus anorganischem Phosphat bestand. Man kann aber die Möglichkeit nicht in Abrede stellen, dass ein Austritt organischen Phosphates und eine Vergärung von Hefeglykogen dazu beigetragen haben können, dass dasselbe Defizit der Gärprodukte hier nicht beobachtet werden konnte, wie es im Versuche mit Acetondauerhefe in Tab. 3 dargestellt wurde.

6. Isolierung kristallisierter Trehalose nach Vergärung von Glucose

Die Trehalosewerte, die aus der Zunahme des Reduktionswertes und aus der optischen Aktivität berechnet wurden, stimmten meistens gut überein. Wie im Vorgehenden gesagt, ist es ELANDER und MYRBÄCK (10) gelungen, Trehalose aus Gärmischungen mit Mazerationssaft zu isolieren. Vieles sprach daher dafür, dass der bei der Gärung mit Acetondauerhefe als Trehalose angegebene Zucker tatsächlich aus Trehalose bestand. In drei Gärversuchen, wo das Fermentsystem in einem aus Acetondauerhefe und in zwei aus gewaschener Hefe und Kochsaft bestand, wurden Versuche angestellt, die analysierte Trehalose zu isolieren, um die Identität des Zuckers eindeutig festzustellen. Die Ergebnisse sind in Tab. 12a und 12b dargestellt. Die Zusammensetzung der Gärproben ist im experimentellen Teil angegeben. In den Proben 2 und 3 war jedoch die Glucosemenge 150 mg statt 100 mg pro Gärprobe von 2 ml. Die Phosphatkonzentration war in allen drei Proben 0,05 M. Proben 1 und 3 wurden aus 60 und Probe 2 aus 50 Gärproben zusammengesetzt. Freie Glucose wurde mit Presshefe beseitigt. Die Proben wurden mit Quecksilbersulfat enteiweisst. Die Ausbeute der Proteinzentrifugate wurde aus den erhaltenen Volumennungen berechnet und betrug 66, 59 und 56 % der ursprünglichen Gärproben. In Probe 1 wurde Trehalose sowohl aus der Zunahme des Reduktionswertes, als auch aus der optischen Aktivität, in den beiden übrigen nur nach der letzteren Methode bestimmt. HMP wurde mit Bariumacetat und 75 % Alkohol entfernt. Die Proben wurden bis auf $\frac{1}{3}$ des Volumens der Proteinzentrifugate eingengt und die Ba-Ionen mit Schwefelsäure beseitigt.

Tab. 12 a. Neubildung der Trehalose in den Isolierungsversuchen.

Glucose und Trehalose + 2 H₂O in g berechnet.

Nr.	Gärzeit, Min.	Zugesetzte Glucose	Vergor. Glucose	Trehalose		Preformierte Trehalose	Neugebildete Trehalose	Trehalose in % zugesetzter Glucose
				aus Zunahme des Red.-Wertes	aus opt. Aktivität			
1	180	6,0	2,41	2,49	2,60	1,04	1,56	24,8
2	370	7,5	4,25		1,80	0,10	1,70	21,6
3	330	9,0	5,16		2,57	0,12	2,45	25,9

Tab. 12 b. Ausbeute kristallisierter Trehalose.

Trehalose wie in der vorigen Tabelle berechnet.

Nr.	Menge der Trehalose in Krist.-Proben	Auskrist. Trehalose	Ausbeute in %	Schmelzpunkte nach Umkrist.	c g/100 ml Lösung	α_D in 2 dm-Rohr	$[\alpha]_D^{20}$
1	1,56	1,14	73	95–96°	2,23	+ 7,94	+ 178,0°
2	0,93	0,71	76	95–96°	2,22	+ 7,90	+ 177,9°
3	1,02	0,80	78	94–95°	2,25	+ 8,00	+ 177,8°

Durch Kochen mit Knochenkohle und Filtrieren durch Kieselgur wurden die Proben von missfarbigen Substanzen befreit. Die Proben durchliefen danach Kationen- und Anionenaustauscher, wie im experimentellen Teile beschrieben worden ist. Die Trehalosekonzentration der in dieser Weise behandelten Proben ist in Tab. 12b dargestellt. Die Proben wurden dann bis zur Sirupkonsistenz eingengt und mit Alkohol bis auf 82–85% verzetzt und zwecks Kristallisation in den Kühlschrank gebracht. Beim Alkoholzusatz entstanden mehrmals flockige Niederschläge, die abfiltriert werden mussten. Die ausgeschiedenen Kristalle waren farblos und besaßen das für Trehalose bezeichnende Aussehen. Aus Tab. 12b geht hervor, dass die Ausbeute der Trehalose 73–78% der aus der optischen Aktivität berechneten Werte ausmachte. Die Verluste beim Filtrieren der Proben vor der Kristallisation waren darin nicht einbezogen. In Tab. 12b sind auch die Schmelzpunkte und die spezifischen Aktivitäten der Proben nach Umkristallisieren aus 85%igem Alkohol eingetragen. Nach MYRBÄCK (3) ist der Schmelzpunkt der Trehalose mit 2 Kristallwassern 96°. Nach SCHUKOW (26) ist, wie früher gesagt, die spezifische Aktivität des Dihydrates +178,3°.

In Probe 2 wurde der Alkohol abdestilliert und die optische Aktivität der Mutterlauge bestimmt. Sie entsprach 172 mg Trehalose. Diese Menge zusammen mit der auskristallisierten Trehalose machte 95% derjenigen der ursprünglichen Kristallisationsprobe aus. Die Mutterlauge enthielt 7,43 mg organischen Phosphates, wovon ein Teil wahrscheinlich aus nicht ausgefälltem THP bestand. Dieser Wert gibt jedoch nicht die ursprüngliche Konzentration dieses Esterphosphates in der Gär-

probe an, da die Konzentration durch die angebrachten Reinigungsverfahren reduziert wurde.

Es geht aus den drei Kristallisationsversuchen eindeutig hervor, dass die bei der Acetonhefegärung gebildete nicht reduzierende Substanz von hoher optischer Aktivität hauptsächlich aus Trehalose bestand.

7. Einwirkung von Arsenat auf die Synthese der Trehalose

HARDEN und YOUNG (39) fanden 1910, dass die Gärungsaktivität des Hefepresssaftes für verschiedene Substrate wie Glucose, Fructose und für die Phosphorsäureester dieser Zucker beträchtlich von Arsenat aktiviert wurde. HDP, das nur langsam von diesem Fermentsystem angegriffen wird, wurde in Gegenwart von Arsenat ebenso schnell wie Glucose vergoren.

In den dreissiger Jahren des 20. Jahrhunderts wurde die Arsenataktivierung der Gärung und Glykolyse eingehend untersucht. Es ging aus diesen Untersuchungen hervor, dass die Oxydation des Triosephosphates mit einer Veresterung anorganischen Phosphates eng verbunden war. Arsenat konnte zwar Phosphat bei der Oxydation ersetzen, konnte aber nicht in veresterter Form nachgewiesen werden. Die hervorragendsten dieser Untersuchungen wurden von MEYERHOF und Mitarbeitern (40, 41, 42, 43) und von NEEDHAM und PILLAI (44, 45) ausgeführt. 1939 gelang es WARBURG und CHRISTIAN (46), das Oxydationsferment der Gärung aus Hefe zu isolieren und in kristallisierter Form herzustellen. Mit dem reinen Oxydationsferment konnten sie zeigen, dass die Oxydation in Gegenwart anorganischen Phosphates eine Gleichgewichtsreaktion zwischen Glycerinaldehydphosphorsäure und anorganischem Phosphat einerseits und 1,3-Diphosphoglycerinsäure andererseits war. 1,3-Diphosphoglycerinsäure wurde von NEGELEIN und BRÖMEL (47) isoliert. Das Ferment, das die Phosphorsäure in der Stellung 1 der 1,3-Diphosphoglycerinsäure auf ADP übertrug, wurde von BÜCHER (48) aus Hefe hergestellt. Die Untersuchung von WARBURG und CHRISTIAN zeigte weiter, dass Glycerinaldehydphosphorsäure in Gegenwart von Arsenat statt Phosphat vollkommen in 3-Phosphoglycerinsäure, ohne gleichzeitige Veresterung des ADPs, überführt wurde. WARBURG nimmt jedoch an, dass eine labile Arsenophosphoglycerinsäure zuerst gebildet wird, dass diese aber von selbst in 3-Phosphoglycerinsäure und Arsenat zerfällt.

Der von WARBURG aufgeklärte Oxydationsmechanismus konnte jedoch keine Antwort auf die Frage geben, warum die Vergärung von HDP in Gegenwart von Arsenat bei 2-Phosphoenolbrenztraubensäure durch Mangel an Phosphatacceptor nicht gebremst wurde, sondern ebenso schnell verlief, als wäre Glucose das Substrat. MEYERHOF und JUNOWICZ-KOCHOLATY (49) konnten jedoch einige Jahre später zeigen, dass die Dephosphorylierung der 2-Phosphoenolbrenztraubensäure indirekt durch Arsenat aktiviert wurde. Da ATP zur Veresterung neuer Glucose nicht verbraucht wird, nimmt seine Konzentration während der Arsenatgärung immer zu, während ADP, das für die Dephosphorylierung der Phosphoenolbrenztraubensäure notwendig ist, verschwindet. Das hat zur Folge, dass die Konzentration der 2-Phosphoenolbrenztraubensäure zunimmt und das Gleichgewicht zwischen 3-Phosphoglycerinsäure, 2-Phosphoglycerinsäure und 2-Phosphoenolbrenztraubensäure gegen Zurückbildung der Erstgenannten verschoben wird. Durch den Überschuss an ATP wird dieser Ester zu 1,3-Diphosphoglycerinsäure phosphoryliert, die mit der reduzierten Glycerinaldehydphosphatdehydrogenase unter Bildung von Glycerinaldehydphosphorsäure und anorganischem Phosphat reagiert. Das gleichzeitig aus ATP

entstandene ADP kann neue 2-Phosphobrenztraubensäure dephosphorylieren. Da jedoch die Oxydation in Gegenwart von Arsenat schneller als übrige Reaktionen verläuft, geht die Gesamtreaktion gegen einen ebenso schnellen Abbau der Phosphoenolbrenztraubensäure, als wäre Glucose das Substrat.

Da die Bildung eines Überschusses von ATP bei der Gärung in Gegenwart von Arsenat verhindert wird, müssen dadurch auch energieverlangende Prozesse gehemmt werden. Wie schon in der Einleitung erwähnt, fanden SUSSMAN und SPIEGELMAN (17), dass bei der anaeroben Vergärung der Glucose mit *Saccharomyces cerevisiae* Assimilation von Kohlehydraten und Stickstoff und Enzymadaptation durch Arsenat gehemmt werden. Die Arsenathemmung kann ganz oder teilweise durch Zusatz von Phosphat reversibel gemacht werden. Allem Anschein nach wirkt also Arsenat in derselben Weise auf die Gärung der lebenden Zellen wie auf die aus Hefe isolierten Gärungsfermente ein.

In dieser Untersuchung über Trehalosebildung bei Vergärung von Glucose mit Acetonhefe hat sich die Bedeutung der Bildung energiereichen Phosphats darin gezeigt, dass die neugebildete Trehalose in einem beinahe konstanten Verhältnis zu gleichzeitig vergorener Glucose stand. Wenn die Gärung aufhörte, dann hörte auch die Synthese der Trehalose auf. Aller Wahrscheinlichkeit nach wird diese Synthese durch Arsenat gehemmt. Wenn man eine Arsenathemmung in einem Gärssystem wie in dem hier verwendeten nachweisen will, liegt eine Schwierigkeit darin, dass die Konzentration des anorganischen Phosphats verhältnismässig gross sein muss, um die Gärung in Gang zu bringen. Demzufolge wird Phosphat bei der Oxydation erfolgreich mit Arsenat konkurrieren können. Die Hemmung kann deswegen erwartungsgemäss nie so vollständig werden, als wenn Zusatz von Phosphat entbehrlich wäre, wie es in den Versuchen von SUSSMAN und SPIEGELMAN mit lebender Hefe der Fall war.

Wie aus den in Fig. 2 und 3 dargestellten Gärversuchen hervorgeht, war 0,05 *M* Phosphat die niedrigste Phosphatkonzentration, die einen vollständigen Umsatz zugesetzter Glucose ermöglichte. In einem Gärungsversuch wurde geprüft, ob in Gegenwart von Arsenat eine niedrigere Phosphatkonzentration als 0,05 *M* verwendet werden konnte. Aus Fig. 5 geht jedoch hervor, dass die Gärung in Gegenwart von 0,025 *M* Phosphat sehr gehemmt war (Kurve 1). Beim Zusatz von 5×10^{-3} *M* Arsenat wurde die doppelte Kohlensäuremenge entwickelt, nach etwa 1 Stunde aber hörte die Gärung vollkommen auf (Kurve 2). Auch in Gegenwart von Arsenat war also 0,05 *M* Phosphat die niedrigste Konzentration, die einen vollständigen Umsatz zugesetzter Glucose gestattete. Bei dieser Phosphatkonzentration wurde die Einwirkung von 5×10^{-4} und 5×10^{-3} *M* Arsenat auf die Vergärung von Glucose untersucht (Kurve 4 und 5). In Gegenwart von 5×10^{-4} *M* Arsenat unterschied sich der Verlauf der Gärung kaum vom Versuche ohne Arsenat. Etwas mehr Kohlensäure hatte sich jedoch gebildet, wenn die Gärgeschwindigkeit abzunehmen begann (Kurve 3 und 4). Durch 5×10^{-3} *M* Arsenat wurde die Gärgeschwindigkeit stark aktiviert und war beinahe ebenso gross wie in Gegenwart von 0,15 *M* Phosphat (Kurve 6, Fig. 2). Eine grössere Substratmenge wurde jedoch in Gegenwart von Arsenat vergoren, was dadurch verursacht werden konnte, dass die Konzentration phosphorylierter und in Trehalose umgewandelter Glucose am Ende der Gärung niedriger war. Es war aber auch möglich, dass Abbau und Vergärung des Hefeglykogens durch Gegenwart von Arsenat stimuliert wurde.

Die Neubildung von Trehalose wurde in Gärproben in Gegenwart von 0,05 *M* Phosphat und 5×10^{-4} und 5×10^{-3} *M* Arsenat bestimmt (Tab. 13). Doppelpuben

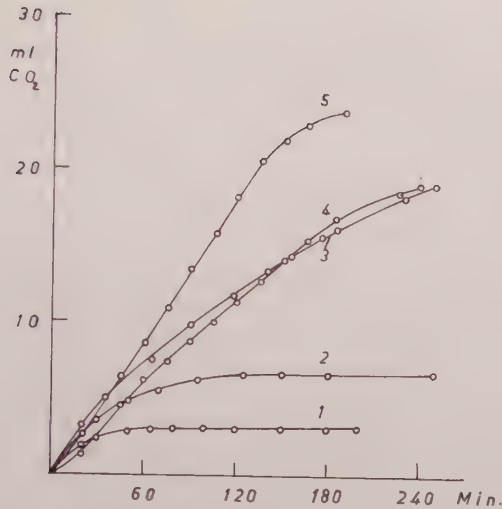


Fig. 5. Einwirkung von Arsenat auf die Glucosegärung mit gewaschener Acetonhefe.

1. Zusatz von 0,025 M Phosphat.
2. » » 0,025 M » und 5×10^{-3} M Arsenat.
3. » » 0,05 M » »
4. » » 0,05 M » und 5×10^{-4} M Arsenat.
5. » » 0,05 M » und 5×10^{-3} M Arsenat.

Tab. 13. Einwirkung des Arsenates auf die Synthese der Trehalose.

In den Proben 1-4 war die Konzentration des Arsenates 5×10^{-4} M und in den Proben 5-8 5×10^{-3} M. Alle Werte als mg Glucose ausgedrückt.

Nr.	Gärzeit, Min.	Mit gewaschener Hefe vergorene Glucose	Reduzierende Substanzen		Diff.	Hydrolysegrad der Trehalose in %	Nach Korr. unvollst. Hydrolyse	Neugeb. Trehalose in % vergorener Glucose	Hemmung der Synthese in %
			vor Hydrolyse	nach Hydrolyse					
1	163	56,0	1,6	31,4	29,8	82,0	36,3	56,4	
2	»	»	1,5	30,0	28,5	»	34,8	53,7	
3	245	75,0	1,0	32,2	31,2	»	38,0	44,4	
4	»	»	1,1	31,7	30,6	»	37,3	43,5	
5	100	56,0	11,3	29,2	17,9	»	21,8	30,5	44,1
6	»	»	13,1	31,2	18,1	»	22,1	31,1	43,2
7	190	89,9	1,0	24,1	23,1	»	28,2	26,1	23,2
8	»	»	1,0	24,5	23,5	»	28,7	26,7	21,6

wurden zu zwei Zeitpunkten ausgenommen, a) wenn 15 ml Kohlensäure pro Gärprobe entwickelt worden waren, d. h. zu der Zeit, wenn freie Glucose in den Gärproben ohne Arsenat verbraucht worden war, und b) wenn die Gärgeschwindigkeit so viel abgenommen hatte, dass die Gärung als beendet betrachtet werden konnte. Dieser Zeitpunkt war jedoch, wie früher gezeigt worden ist, schwer

festzustellen, da durch dieses Fermentsystem allmählich sowohl Trehalose als auch Phosphorsäureester abgebaut und vergoren wurden. Bei den Arsenatgärungen wurde dasselbe Fermentsystem wie bei den Versuchen in Tab. 7 verwendet. Freie Glucose wurde nicht mit Presshefe beseitigt, sondern die Gärproben wurden sofort nach der Gärung mit Bariumhydroxyd und Zinksulfat enteiweisst und Trehalose aus der Zunahme des Reduktionswertes in den Proteinfiltraten bestimmt. Der Gehalt an Trehalose in Hefe und Kochsaft geht aus Tab. 7 hervor. Dort ist auch die Menge Trehalose wiedergegeben, die mit 0,05 *M* Phosphat ohne Arsenat neugebildet wurde (Probe 4, Tab. 7).

Wenn 15 ml Kohlensäure entwickelt worden waren, hatte sich in Gegenwart von 5×10^{-4} *M* Arsenat ebenso viel Trehalose wie in der Probe ohne Arsenat gebildet. Die Trehalosesynthese wurde demnach von dieser Arsenatkonzentration nicht gehemmt. In den am Ende der Gärung herausgenommenen Proben hatte die Konzentration der Trehalose um 2 mg zugenommen. Eine ähnliche Zunahme der Trehalose, nachdem freie Glucose in den Gärproben verbraucht worden war, fand auch, wie im Vorgehenden gezeigt wurde, in Proben ohne Arsenat statt (Probe 11, Tab. 2).

In Gegenwart von 5×10^{-3} *M* Arsenat war die Synthese der Trehalose in den Proben, die bei 15 ml entwickelter Kohlensäure herausgenommen wurden, bis auf 43,6% gehemmt. Aus einem Vergleich der direkten Reduktionswerte der Proben 1 und 2 einerseits und Proben 5 und 6 andererseits geht hervor, dass die Konzentration der freien Glucose der letztgenannten Proben etwa 10 mg grösser war. Dies Verhältnis zeigte, dass die Phosphorylierung der Glucose durch 5×10^{-3} *M* Arsenat gehemmt wurde, und es muss für sehr wahrscheinlich gehalten werden, dass dies der Grund der niedrigeren Synthese der Trehalose war. Wenn die Gärung der Arsenatproben aufhörte, hatte die Trehalosekonzentration dieser Proben um weitere 6,4 mg zugenommen. Die Arsenathemmung sank deshalb bis auf 22,4% herab. Zu diesem Zeitpunkt war das Verhältnis zwischen neugebildeter Trehalose und vergorener Glucose nur 26,4%, während dasselbe Verhältnis in der Probe ohne oder mit 5×10^{-4} *M* Arsenat etwa 55% war. Daraus ist ersichtlich, dass die synthetisierende Wirkung des Fermentes in Gegenwart von 5×10^{-3} *M* Arsenat erheblich geringer wurde. Diese Arsenatkonzentration konnte jedoch nicht verhindern, dass eine Phosphorylierung stattfand, die gross genug war, um eine beträchtliche Trehalosebildung zu gestatten. Es ist möglich, dass eine grössere Arsenatkonzentration eine stärkere Hemmung der Synthese hervorrufen könnte.

Am Ende der Gärung war die Summe der vergorenen und der in Trehalose umgewandelten Glucose bei 5×10^{-4} *M* Arsenat 108 mg und bei 5×10^{-3} *M* Arsenat 113,6 mg. Wie aus den Untersuchungen von HARDEN und von MEYERHOF hervorgeht, wird HDP von Hefesaft in Gegenwart von Arsenat ebenso schnell wie Glucose vergoren. Wahrscheinlich gab es deswegen am Ende der Gärung bei 5×10^{-3} *M* Arsenat nur eine geringe Menge veresterter Glucose. Diese Konzentration wurde jedoch nicht festgestellt, weil Arsenat auf die kolorimetrische Phosphatbestimmung einwirkte. Die Gesamtmenge umgesetzter Glucose in Gegenwart von 0,05 *M* Phosphat betrug 103 mg (Tab. 8). War 5×10^{-3} *M* Arsenat zugegen, war die Konzentration der umgesetzten Glucose um etwa 10 mg grösser, obwohl keine veresterte Glucose darin eingerechnet wurde. Dies kann kaum in anderer Weise erklärt werden, als dass ein Teil des Hefeglykogens vergoren und möglicherweise auch in Trehalose umgewandelt wurde. DOUDOROFF, BARKER und HASSID (50) haben gezeigt, dass durch eine Phosphorylase aus *Pseudomonas saccharophila* freie Glucose aus Rohrzucker in Gegenwart von Arsenat gebildet wird. Die Reaktion ist als Arsenolyse bezeichnet worden, und es

wird angenommen, dass ein labiler Arsensäureester gebildet wird, der von selbst zerfällt. Es ist auch von KATZ, HASSID und DOUDOROFF (51) gezeigt worden, dass beim Abbau der Stärke von Kartoffelphosphorylase eine Arsenolyse stattfindet. Es scheint deshalb möglich, dass auch in acetonetrockneter Hefe eine Arsenolyse des Hefeglykogens unter gewissen Bedingungen stattfinden kann.

Diskussion

In einer Untersuchung des anaeroben Glucoseumsatzes der Bäckerhefe fanden TREVELYAN *et al.*, dass 70% zugesetzter Glucose zu Kohlensäure und Alkohol vergoren wurden, während 15% in Zellkohlehydrate umgewandelt wurden. Das neugebildete Kohlehydrat bestand vorwiegend aus Glykogen, jedoch konnte auch eine geringfügige Synthese von Trehalose nachgewiesen werden. In der in diesem Aufsatz dargestellten Untersuchung über den Glucoseumsatz der acetonetrockneten Bäckerhefe ist gezeigt worden, dass 25–35% zugesetzter Glucose in Trehalose umgewandelt wurden. Aus den Berechnungen der Gesamtmenge umgesetzter Glucose ging nicht hervor, dass sich, ausser Kohlensäure und Alkohol, andere Umsatzprodukte als Phosphorsäureester und Trehalose bildeten.

NILSSON und ALM (12) vermuteten, dass die desorganisierende Wirkung des Acetons auf den Gärverlauf der Acetonunterhefe eher darauf beruht, dass Aceton von den Zellen aufgenommen worden ist, als darauf, dass für die Gärung wichtige Substanzen extrahiert worden sind. Die gegenwärtige Untersuchung hat gezeigt, dass der Gärverlauf der Acetonoberhefe, nach Auswaschen und erneutem Trocknen der Hefezellen, nicht verändert wurde. Da in den Zellen zurückgebliebenes Aceton in dieser Weise sicher entfernt wurde, muss der Grund der Veränderung des Gärungsverlaufes der frischen Bäckerhefe durch Aceton ein anderer sein.

Die unvollständige Vergärung der Glucose und die Anhäufung der Phosphorsäureester, die den Gärverlauf gewisser Trockenhefepräparate charakterisieren, entstehen nach MEYERHOF (38) dadurch, dass die Adenylpyrophosphatase teilweise inaktiviert ist. Durch Zusatz von Adenylpyrophosphatase zu derartigen Fermentsystemen konnte MEYERHOF eine ebenso vollständige Vergärung der Glucose wie mit lebender Hefe hervorrufen.

Die Anhäufung phosphorylierter Glucose, die nach MEYERHOF durch die herabgesetzte Aktivität der Adenylpyrophosphatase entsteht, würde in Acetondauerhefe den Anlass einer gesteigerten Synthese der Trehalose geben können. Es ist jedoch anzunehmen, dass auch ein anderer Faktor bei der in diesem Fermentsystem dominierenden Trehalosesynthese eine wesentliche Rolle spielen kann. Durch die Behandlung mit Aceton und Äther verändern sich die Hefezellen im Vergleich mit denen der frischen Hefe in charakteristischer Weise. Die Form der Acetonhefzellen ist unregelmässig. Sie sehen wie eingeschrumpft aus. Bei pH 4,6 dringt Methylenblau sofort in die Zellen ein. Phosphorsäureester und Trehalose werden leicht mit Wasser bei Zimmertemperatur aus den Zellen extrahiert. Diese Eigenschaften deuten darauf hin, dass die Permeabilität der acetonbehandelten Zellen sich derartig verändert hat, dass sie eine entscheidende Einwirkung auf deren Stoffwechsel haben kann. Es lässt sich denken, dass Verschiebungen der Gleichgewichtsverhältnisse an denjenigen Fermenten eintreten können, die in der Entstehung von permeablen Substanzen beteiligt sind und dass diese Gleichgewichtsverschiebungen auf die Zusammensetzung der Gärprodukte einwirken können. Beim Trocknen der Hefe fand PAYEN (5), dass Glykogen in Trehalose umgewandelt wurde. Von TREVELYAN *et al.* (9) und von CHUNG

und NICKERSON (52) wird es angenommen, dass G-1-P ein gemeinsames Zwischenprodukt bei den Synthesen von Glykogen und von Trehalose ist. Wenn ein Gleichgewichtsverhältnis zwischen Glykogen und Trehalose vorliegt, dann überwiegt in der frischen Hefe die Synthese des Polysaccharides Glykogen während in der acetontrockneten Hefe zufolge Permeabilitätsveränderungen nur das leichtlösliche Disaccharid Trehalose gebildet werden kann.

In zwei von drei Versuchen, in denen die Trehalosebildung während mehrerer Stunden verfolgt wurde, stellte es sich heraus, dass das Verhältnis zwischen neugebildeter Trehalose und vergorener Glucose beinahe konstant war. Dies spricht dafür, dass das Disaccharid aus phosphorylierter Glucose gebildet wird. Arsenat, das die Bildung von ATP bei der anaeroben Gärung verhindert, wirkte in $5 \times 10^{-3} M$ Konzentration auf die Trehalosesynthese stark hemmend ein, was ferner die Bedeutung der phosphorylierten Zwischenprodukte bei der Synthese bestätigte.

Mit Bariumacetat und 75% Alkohol wurde gleichzeitig mit HMP ein nichtreduzierendes, teilweise unbekanntes Esterphosphat gefällt. Die nicht gefällte Esterfraktion enthielt unbekanntes Esterphosphat, das bei der Säurehydrolyse etwa dieselbe Stabilität zeigte, wie die von ROBISON und MORGAN für THP gefundene. In dem bei den Gärversuchen mit gewaschener Hefe verwendeten Kochsaft kam ein Esterphosphat vor, das dieselben Eigenschaften wie das Esterphosphat der Trehalosefiltrate in den Gärversuchen besass. Die Konzentration war jedoch etwas niedriger. Der Konzentrationsunterschied war aber nicht so gross, dass man mit Sicherheit die Möglichkeit ausschliessen konnte, dass das Esterphosphat im Kochsaft vorausgebildet vorkam. Ein entscheidender Beweis dafür, dass THP sich während der Glucosegärung mit Acetonhefe bildet, ist folglich noch nicht erhalten worden. Mit Hilfe papierchromatographischer Analysen der beiden Esterfraktionen hofft der Verfasser, weitere Auskunft über die Zusammensetzung der Phosphorsäureester bei der Acetonhefegärung zu gewinnen.

Zusammenfassung

1. Mit acetontrockneter Oberhefe und mit einer derartigen Hefe, die durch Auswaschen von Trehalose befreit worden war, ist bei der Glucosegärung die Einwirkung verschiedener Phosphatkonzentrationen auf den Gärverlauf untersucht worden. Der Gärverlauf zeigte dieselben Eigenschaften wie die von NILSSON und ALM hergestellte acetontrocknete Unterhefe.

2. Nach 4,5 Stunden Gärung wurde mit acetontrockneter Oberhefe in 0,05 *M* Phosphat ein Vergleich angestellt zwischen Kohlensäureentwicklung und der Neubildung von Trehalose in Luft und in Stickstoff. Kein Unterschied der analysierten Werte konnte beobachtet werden und daraus wurde der Schluss gezogen, dass der Stoffwechsel der Acetondauerhefe auch in Luft hauptsächlich anaerob ist.

3. In zwei Versuchen wurde nach 6 Stunden Gärung die Neubildung der Trehalose ohne und in Gegenwart von $2 \times 10^{-3} M$ Natriumfluorid verglichen. Ohne Fluorid wurden 35% zugesetzter Glucose in Trehalose umgewandelt. Mit Fluorid waren sowohl Gärung als auch Trehalosebildung vollständig gehemmt.

4. Der Umsatz der Trehalose wurde in zwei Versuchen während 24 Stunden und in einem Versuch, bis die Vergärung zugesetzter Glucose aufhörte, verfolgt. Die Phosphatkonzentration war 0,05 *M*. In zwei von den drei Versuchen war während der Gärperiode das Verhältnis zwischen neugebildeter Trehalose und vergorener Glucose beinahe konstant und betrug in dem einen Versuche 50–60% und im anderen

40–47%. Der dritte Versuch zeigte ein abweichendes Verhältnis in der Beziehung, dass die Geschwindigkeit der Trehalosesynthese am Anfang der Glucosegärung erheblich grösser war als nachher.

5. Hörte die Glucosegärung der im Punkt 4 erwähnten Versuche auf, so liess die Neubildung der Trehalose nach. Allmählich fing eine Hydrolyse des Disaccharides an, die sich bei Gärung mit Acetondauerhefe in der Bildung einer mit der hydrolysierten Trehalose äquivalenten Menge Glucose zeigte. Mit gewaschener Hefe wurde diese Glucose wieder vergoren.

6. In den im Punkt 4 erwähnten Versuchen wurde der Phosphatumsatz während 24 Stunden verfolgt. Nach etwa 3 Stunden liess die Gärgeschwindigkeit nach. Die Konzentration veresterten Zuckers wurde dadurch niedriger. Gleichzeitig nahm auch die Geschwindigkeit der Trehalosesynthese ab, was auf einen Zusammenhang phosphorylierter Glucose und synthetisierter Trehalose deutete.

7. Versuche wurden ausgeführt, die Konzentration des THPs aus dem Unterschied nicht reduzierenden und leicht hydrolysierbaren Esterphosphats zu berechnen. Die Wahrscheinlichkeit dafür, dass dieser Ester ein Zwischenprodukt bei der Trehalosesynthese ist, wurde besprochen.

8. Die Synthese der Trehalose bei der Glucosegärung in Gegenwart von 0,10 und 0,15 *M* Phosphat wurde mit der bei 0,05 *M* Phosphat verglichen. Der Maximalwert neugebildeter Trehalose war von der Konzentration des Phosphates unabhängig. In Gegenwart von 0,10 *M* Phosphat wurde aber die Synthese erheblich verzögert, was möglicherweise mit der erhöhten Konzentration des anorganischen Phosphates zusammenhing.

9. In zwei Versuchen mit gewaschener Hefe wurde nach 4 Stunden Gärung die Gesamtkonzentration vergorener, veresteter und in Trehalose umgewandelter Glucose bestimmt. Die Gesamtmenge stimmte gut mit der berechneten Substratmenge überein. Dies war aber in einem Versuch mit Acetondauerhefe nicht der Fall. Es wurde hier befürchtet, dass sich ein Teil der Umsatzprodukte den Analysen entzogen hatte.

10. Kristallisierte Trehalose ist aus drei Gärversuchen, einem mit Acetondauerhefe und zwei mit gewaschener Hefe, isoliert worden. Die isolierte Menge entsprach 73–78% der Trehalosemenge, die aus der optischen Aktivität der Proben berechnet werden konnte.

11. Einwirkung von 5×10^{-4} und 5×10^{-3} *M* Arsenat wurde auf die Synthese der Trehalose untersucht. Die Phosphatkonzentration war dabei 0,05 *M*. In Gegenwart von 5×10^{-3} *M* Arsenat war die Synthese im Verhältnis zu einer Probe ohne Arsenat bis auf 40% gehemmt. Dieselbe Menge Glucose war dabei in beiden Proben vergoren. Die Hypothese, dass Trehalose aus phosphorylierter Glucose aufgebaut wird, wurde durch die Arsenathemmung gestützt.

12. Der Anlass dazu, dass bei der Glucosegärung mit acetonetrockneter Hefe nicht Glykogen, sondern Trehalose synthetisiert wird, ist besprochen worden.

Herrn Professor Dr. K. MYRBÄCK spreche ich für sein Interesse und seine anregende Kritik meinen aufrichtigsten Dank aus. Der Universität zu Stockholm bin ich für die Gewährung eines Doktorandstipendiums zu grossem Dank verpflichtet.

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Studies on Enterokinase

II. The further purification

By IKUO YAMASHINA¹

In a previous paper (1), the partial purification and the general properties of enterokinase were reported. The best samples of enterokinase, containing 630 Kunitz' units per mg, thus being 15 times stronger than those of Kunitz, separated into two fractions in the Tiselius electrophoresis apparatus, one of them carrying all of the activity. In this paper a procedure is described by means of which an activity of 2600 units per mg has been obtained. It includes some modifications of the earliest steps in the procedure of Kunitz and some essential changes in the final fractionation with organic solvents in the cold in the presence of salts. Short communications about this procedure have been given on two different occasions (2, 3).

Experimental

The enterokinase activity has been determined as described in the previous paper.

Preparation of the starting material

As raw material, frozen swine duodenal fluid contents were used. Although Kunitz obtained a nearly 4000 times purification with fairly good yield (4), his procedure is not particularly easy to apply. Since the enterokinase is stable against the action of proteolytic enzymes, the duodenal fluid contents can be submitted to digestion by the proteolytic enzymes present in the same fluid.

For this purpose the fluid contents were diluted with three volumes of tap water and left for digestions at room temperature in the presence of toluene for two days, the pH being maintained at 9 by the occasional addition of 5N NaOH. The proteolytic enzymes present are capable of digesting the protein impurities, no additional action being observed when glycerol extracts of the pancreas and of the intestinal mucosa were added. After the digestion, the pH was lowered to 4.0 with 5N H₂SO₄, 50 g of Hyflo Super-Cel was added to every liter and the suspension was filtered through cheese-cloths. The filtration procedure runs much easier than without previous digestion and clear filtrates are obtained. The pH was raised to 8.0 by the addition of 5N NaOH and the filtrate was submitted to ammonium sulphate fractionation.

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Solid ammonium sulphate was added until 0.8 saturation followed by the addition of 4.0 ml a 0.4*M* of borate buffer, pH 9.0 to every liter, the pH being adjusted to 8.0. The flocculent precipitate rising to the surface were collected, dissolved in water and fractionated again by ammonium sulphate. The fraction between 0.4 and 0.8 saturation was collected, dissolved in water, dialyzed in a shaking apparatus against distilled water at +5° C during 2 days and lyophilized.

Thus, enterokinase preparations having 100 units per mg dry substance, instead of 40 as found by Kunitz (4), were obtained. The yield was also higher, 1 g of dry substance being obtained from 1 kg of frozen duodenal fluid contents. This preparation can be stored in the cold room at -10° C, at least for several months without any loss of activity. It was used as the starting material for the further purification studies.

Further purification

The changes introduced in the method of preparing the starting material necessitated changes in the further purification procedures.

Thus, ethanol fractionation at pH 4.7 as described in the previous report was not adopted because of the viscosity of the present starting material, which made the separation in the centrifuge of the precipitates from the supernatant difficult. Therefore, ethanol fractionation in the presence of 5 % of calcium acetate at pH 4.5 was adopted. This principle has earlier been applied for the separation of acidic mucopolysaccharides, e.g. chondroitin sulphuric acid, hyaluronic acid and kerato-sulphuric acid (5).

Ethanol fractionation in the presence of Ca^{++}

The starting material was dissolved in calcium acetate solution to approximately 2 % and the pH was adjusted to 4.5 with 5*N* acetic acid, the final calcium acetate concentration being 5 %. The fractionation was carried out at -5° C using precooled absolute ethanol (all organic solvents were pre-cooled at -10° C). After the removal through centrifugation at -5° C of the viscous substances which precipitate below 47 % ethanol concentration (by volume), the fraction between 47 and 57 % ethanol concentration was collected likewise through centrifugation at -5° C and dissolved in cold water. This product has about 600 enterokinase units per mg protein based on Lowry's colorimetric determination (6). The yield of activity was 80 %.

Acetone fractionation at pH 5.5 in the presence of Zn^{++}

This principle has earlier been applied as described in the previous paper. However, it was found that the presence of ammonium sulphate brings about a better purification mainly because of the increase in the ionic strength of the medium and partly because of the specific action of the ammonium sulphate on the protein molecules. Therefore zinc acetate and ammonium sulphate were added to the aqueous solution of the above mentioned product to a concentration of 0.01*M* and 0.04*M* respectively, the pH being adjusted to 5.5 with acetic acid. The volume was adjusted to 1000 ml for each 20 g of the starting material with 100 units per mg. The fractionation was carried out at 0° C using pre-cooled 50 % acetone (by volume) containing one volume

of acetone and one volume of zinc acetate -acetic acid buffer, pH 5.9 having Zn^{++} in 0.01M.

The acetone fractionation was repeated three times. At the first instance, the fraction between 30 and 45 % acetone concentration was collected and dissolved in 500 ml of cold water. In order to reach an acetone concentration higher than 40% pure acetone had to be used.

In the second fractionation the ammonium sulphate concentration was adjusted to 0.06M, other conditions being exactly the same as before. The fraction between 35 and 45 % acetone concentration was collected and dissolved in 300 ml of cold water. The third fractionation was carried out under the same conditions and the fraction between 37 and 45 % acetone concentration was collected, dissolved in 200 ml of cold water, dialyzed at $+5^{\circ}\text{C}$ during 2 days and lyophilized.

About 250 mg freeze-dried substance having 2600 enterokinase units per mg (4600 units per mg protein) was obtained starting from 20 g of the crude material with 100 units per mg. The yield in activity is about 33 %. Moreover, the metal-combining proteins or glycoproteins which precipitate below 37% acetone concentration during the fractionation procedures and still contain enterokinase activity turn out to be soluble after dialysis against water and can be useful for the recovery of enterokinase.

Purity tests

The preparation thus obtained is 65 times stronger than the best preparation of Kunitz.

In electrophoresis at pH 4.4, the conditions being exactly the same as described in the previous paper (1), a main peak corresponding to the active principle followed by a small one was observed, both of which migrated to the anode.

On ultracentrifugation¹ nearly the same pattern was observed. So far the purity is about 80 %, as calculated by the activity measurement on the main peak separated from the electrophoresis cell.

By paper electrophoresis, where the preparation behaved as a single band, the isoelectric point was estimated to be around 4.0.

Chemical studies

Chemical analyses focussed on the carbohydrate components were performed. Neutral sugars were determined both by paper chromatography using *n*-butanol-acetic acid-water as the developing solvent (7) and by chromatography on a starch column using *n*-butanol-propanol-water as the solvent, according to Gardell's (8) technique.

Fucose, mannose and galactose were detected. The approximate values in percentage of air dry substance are 4, 3 and 8 respectively.

Amino sugars were determined according to the method of Gardell (9). Both glucosamine and galactosamine were present, the amount being 10 % and 3 % respectively.

Thus, the total carbohydrate content of the air-dry preparation with about 10 % moisture amounts to nearly 30 %.

¹ The ultracentrifugation was kindly performed by Dr. A. Ehrenberg of the Medical Nobel Institute, Stockholm (Prof. H. Theorell) to whom the author's thanks are due.

The relation between carbohydrate content and activity

When the carbohydrate content of this preparation is compared with that of one of the earlier preparations having 630 enterokinase units per mg, it is found that there is an increase in the carbohydrate content with the increase in activity, as shown in Table 1. In this table, the activity is expressed in terms of activity per mg protein based on Lowry's colorimetric method and neutral sugars were determined according to the method of Dische as modified by Gurin and Hood (10).

Discussion

A highly active preparation of enterokinase has been obtained. The results of the electrophoresis and ultracentrifugation experiments, which show that the preparation has reached a high degree of purity, together with the findings that the increase in activity is accompanied by an increase in the carbohydrate content, strongly support the idea that enterokinase is a glycoprotein.

Summary

The earlier procedure of preparing and purifying enterokinase has been improved.

Swine duodenal fluid contents were digested by proteolytic enzymes and fractionated by ammonium sulphate under pH control. The material, thus obtained contained 100 enterokinase units per mg, as compared to 40 units per mg as found by Kunitz in 1939. The yield was 100,000 units per kg duodenal fluid content.

The material was purified as follows:

(1) Ethanol fractionation at pH 4.5 in the presence of 5 % of calcium acetate at -5°C .

(2) Acetone fractionation at pH 5.5–5.9 in the presence of Zn^{++} (0.01*M*) an ammonium sulphate (0.04–0.06*M*) at 0°C .

Finally, a preparation having 2600 units per mg was obtained containing 33 % of the total activity of the dry crude starting material with 100 units per mg.

Electrophoresis and ultracentrifugation indicated that the preparation is of a high degree of purity, probably 80 % pure. The chemical analysis showed the presence of the neutral sugars fucose, mannose and galactose and the amino sugars glucosamine and galactosamine.

The total carbohydrate content of the preparation is extremely high, amounting to nearly 30 %.

The conclusion is drawn that the enterokinase is a glycoprotein.

Table 1. Relation between carbohydrate content and activity

Activity/mg protein	1600 units	4600 units
Neutral sugars/protein	10.2 %	19.2 %
Amino sugars/ protein	8.4 %	23.7 %

The author's thanks are due to Professor Erik Jorpes and First Assistant Victor Mutt for their help during the performance of the work. It was made possible through research grants to Professor Jorpes from the Swedish State Medical Research Council, the Swedish manufacturer of insulin, Vitrum Ltd., and the Swedish Cancer Society.

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The normal fatty acids in solid state

A crystal structure investigation

By ERIK VON SYDOW

With 10 figures in the text

Introduction

The normal fatty acids are fundamental components of a great many substances of biological and technical importance, e.g. animal and vegetable fats, lipids, and soaps. Compounds with acids containing an even number of carbon atoms are far more common in nature than those with an odd number, and have in fact been believed to be the only possible ones, but in later years this conception has been revised, e.g. by Hansen, Shorland and Cooke (1).

Exactly one century after the first normal fatty acid was isolated Langmuir (2) published an investigation dealing with, among other things, the properties of surface films of normal fatty acids on water. Langmuir observed that the film thickness was proportional to the number of carbon atoms and arrived at the conclusion that moderately compressed surface films consisted of parallel fatty acid molecules arranged perpendicular to the surface with all the polar carboxyl groups in one plane and in the water, and that the hydrocarbon chains were zig-zag shaped.

Independently of each other de Broglie (3), Piper (4), and Müller (5, 6) in 1923 investigated long chain compounds, the last one normal fatty acids, in solid state using X-ray diffraction. They found that the different specimens gave reflexions caused by widely spaced crystallographic planes and that the distance between these planes, the long spacing, was related to the number of carbon atoms within a homologous series by a linear law. They assumed that in the crystalline state the molecules of long chain compounds were arranged in the same way as in the surface films investigated by Langmuir with the important difference that in the solid state the molecules might exist in dimers and tilted, i.e. they might not necessarily be perpendicular to the planes with the carboxyl groups. This concept has later proved to be correct.

During the following years more than twenty investigations, dealing with long spacing relations have been published, e.g. (7-11). Most of these have been reviewed in *Strukturbericht*. Some of these papers will be referred to later in the text or have been referred to in other papers by the author, but the main features will be given here.

Normal fatty acids exhibit polymorphism. The formation of the different polymorphic forms has been found to be dependent on the temperature, the purity of

the acid and the mode of crystallization. There are three different crystal forms of acids with an even number of carbon atoms called A, B and C, and three or four of odd-numbered acids: A', B', C', (and D'). In each case the long spacing decreases in the order given. For each crystal form the long spacing is a linear function of the number of carbon atoms and the members of one form are supposed to be isomorphous, as the powder diffraction pattern changes very little when passing from one member to the next. The linear functions for the different forms have different slopes and intercepts.

For a crystallographic investigation very pure fatty acids are necessary, as at least the long spacing is affected by the presence of homologous impurities, cf. Francis, Piper and Malkin (9) and Slagle and Ott (12). It is also almost certain that impurities will in some cases affect the crystallization process and give crystal forms, which should not have appeared with a pure acid. The melting points of the normal fatty acids are affected by impurities and the melting point of a sample, as determined in the accurate way suggested by Francis and Piper (11), gives a good idea of its purity. Obviously many of the above mentioned long spacing investigations have been carried out with rather impure fatty acids and the results must in such cases be taken with some reservation. The most accurate melting points are considered to be those given by Francis and Piper (11) and Stållberg-Stenhagen (13).

Müller (14) was the first to investigate the normal fatty acids in solid state in greater detail. By using single crystal X-ray methods he determined the monoclinic unit cell of the B-form of *n*-octadecanoic (stearic) acid and pointed out the main features of the subcell theory discussed later in this paper. Several unit cell determinations carried out with X-ray and electron diffraction have since appeared: the A-forms of *n*-dodecanoic (lauric) (15), *n*-tetradecanoic (myristic) (15) and *n*-octadecanoic acid (16, 17), the B-forms of *n*-hexadecanoic (palmitic) (18) and *n*-octadecanoic acid (14, 16, 17, 19–22), and the C-forms of *n*-dodecanoic (23), *n*-hexadecanoic (18, 19), *n*-octadecanoic acid (16, 17, 22, 24) and *n*-tetracosanoic acid (24 a), the A'-form of *n*-tricosanoic acid (25) and the B'-forms of *n*-heptadecanoic (22) and *n*-tricosanoic acid (24 a, 25). The B- and C-forms of even-numbered fatty acids are monoclinic. The cells of the odd-numbered acids in (22, 25) and of the A-forms of *n*-dodecanoic and *n*-tetradecanoic acid (15) are also said to be monoclinic, but this seems to be wrong.

Schoon (22) made a fruitful suggestion that the monoclinic (tilted) structures of the fatty acids might be deduced from the orthorhombic (vertical) hydrocarbon structure and the vertical arrangement in Langmuir's compressed surface films by translating each chain an integral multiple carbon-carbon distance in the chain direction.

In 1951 Vand, Morley and Lomer (26) published the first crystal structure determination of a normal fatty acid: the C-form of *n*-dodecanoic acid. The result is in good agreement with the general postulates which had been given by some of the authors mentioned above. The structure will be discussed together with the other crystal structures.

In connection with chemical and biological studies and monolayer investigations of many long chain compounds carried out in Professor E. Stenhagen's laboratory at the Institute of Medical Chemistry, University of Uppsala it was found desirable to know more of the solid state of the fundamental long chain compounds, e.g. the normal fatty acids. A general knowledge of the arrangement of long chain molecules will be of great value for the understanding of many important biological and technical processes.

The work dealt with in the present paper and Refs. (27-37) was begun by the author in 1950. Its aim has been to determine the structure of the remaining known crystal forms of the normal fatty acids, to study the formation and existence of the different polymorphs, and to investigate the infrared absorption spectra of the different crystal forms.

Material used

The very pure fatty acids used have been kindly supplied by Professor E. Stenhagen and his collaborators. The preparation has been described in Refs. (13, 27, 38, 39) except for *n*-hendecanoic acid, an impure sample of which was obtained from Columbia Organic Chemicals Inc. The acid was carefully purified via the methyl ester and distilled. The final fractions of the acid had b.p. 119° (0.4 mm) and m.p. 28°0-28°4 C (40).

Methods of crystal structure determination

The different crystal structures have been determined using single crystal X-ray methods. The purpose of the investigations has not been to calculate accurate bond lengths and bond angles between the atoms in the molecules but to reveal the packing of the molecules and possible molecular deformations.

The periodicity in a hydrocarbon chain which is a part of a fatty acid molecule, gives rise to small groups of strong reflexions periodically distributed in the reciprocal lattice of the crystal in question. If the periodicity is distorted in some way these groups will grow in size and the reflexions within them will become less strong. The groups describe a larger reciprocal cell, i.e. a smaller direct cell, which gives the translation between equivalent positions in a chain and in adjacent chains. This cell is called the subcell.

The main advantage of the subcell is its use in structure factor calculations where it was introduced by Müller (14) and later used by Vand (41). By using the subcell concept the structure factor contribution from a long chain can be calculated with the coordinates of only a few atoms, the subcell atoms. This requires a knowledge of the repetition of the subcells, which is a function of *h*, *k* and *l* and the numbers of subcells implied. It is also necessary to know the position of the whole subcell region in the main cell. If the chains are deformed the subcell concept can, of course, not be used.

Patterson projections for the determination of interatomic vectors and electron-density projections, as well as difference syntheses used in the refinement of the structure have been used in the determinations, in all cases carried out along the shortest axis. These calculations have been carried out using Beevers-Lipson (3°) strips, Robertson (3°) strips and the Hägg-Laurent machine (42). The difference syntheses have been of special value in two cases. With very limited intensity material, the errors due to series termination effects have been diminished and when atoms overlap in the projections it has nevertheless been possible to refine their coordinates by using difference syntheses on the two-dimensional data.

Temperature factors, $\exp \left(-B \frac{\sin^2 \theta}{\lambda^2} \right)$, have been applied in three cases and neglected in two cases owing to the limited material in one case and the comparatively

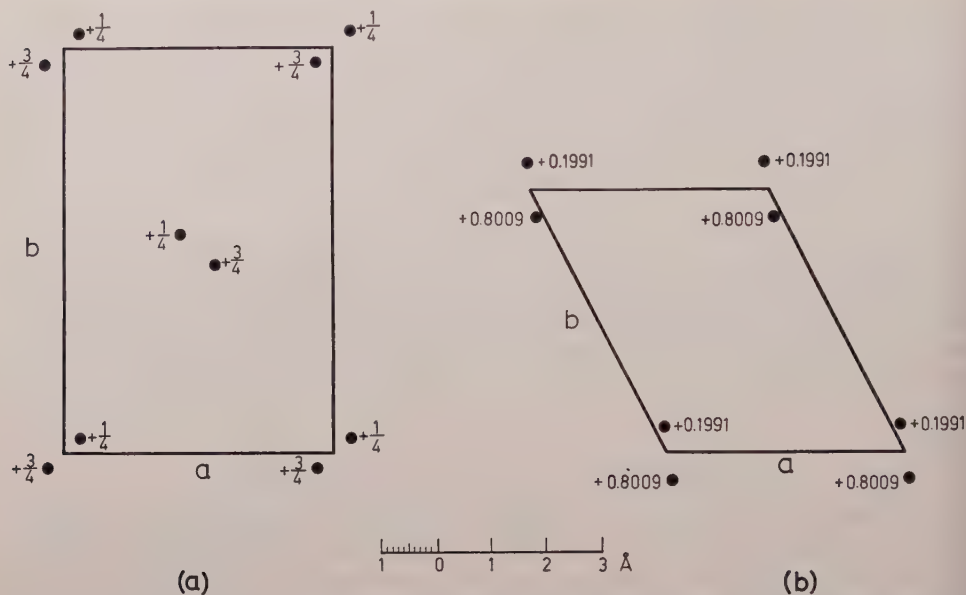


Fig. 1. (a) The orthorhombic subcell with coordinates from Bunn (43). (b) The triclinic subcell with coordinates from Vand and Bell (45).

small B value in the other case. The only disadvantages of neglecting temperature factors in these cases will be poorer agreement between observed and calculated structure factors, and the appearance in the difference syntheses of circular positive ranges around negative areas where the atoms are situated.

The reliability index, $R_1 = \frac{\sum |F_{\text{obs}} - F_{\text{cal}}|}{\sum |F_{\text{obs}}|}$, omitting non-observed reflexions has been calculated in each case and used as a test for the correctness of the structures. The value of this test has been discussed by many authors, but apparently the consensus of opinion is that a structure comprising about equally heavy atoms is correct if $R_1 < 0.20$ with the condition that the deviations are fairly evenly distributed in the material.

Subcell

The subcells, i.e. the cells which describe the repetition in and of the hydrocarbon chains, can be calculated from the distribution of strong reflexions in the reciprocal lattice. There are two kinds of subcells among the normal fatty acids: one orthorhombic and one triclinic. These are the only two types present in covalent normal chain compounds.

The orthorhombic subcell which is shown in Fig. 1 (a) has been analyzed by Bunn (43) using X-rays and Vainshtein and Pinsker (44) using electron diffraction on paraffins. It contains four CH_2 groups.

The cell data are given in Table 1, together with the volumes of the orthorhombic subcells, which have been determined from the reciprocal lattices of the crystal forms having orthorhombic chain packing. In this packing of hydrocarbon chains

Table 1. The dimensions and volume of the orthorhombic subcell.

Long chain compound and crystal form	a_s Å	b_s Å	c_s Å	V per CH_2 Å ³
<i>n</i> -Hydrocarbon Bunn (43)	4.93	7.40	2.534	23.1
<i>n</i> -Hydrocarbon Vainshtein and Pinsker (44)	4.96	7.41	2.54	23.3
<i>n</i> -Octadecanoic acid B-form (31)	4.99	7.40	2.53 ± 0.02	23.3
<i>n</i> -Pentadecanoic acid B'-form (30)	5.02	7.59	2.51 ± 0.02	23.9
<i>n</i> -Dodecanoic acid C-form, Vand <i>et al.</i> (26)	4.97	7.80	2.521 ± 0.007	24.4
<i>n</i> -Hendecanoic acid C'-form (33)	4.92	7.95	2.52 ± 0.02	24.7

Table 2. The dimensions and volume of the triclinic subcell.

Long chain compound and crystal form	a_s Å	b_s Å	c_s Å	α_s	β_s	γ_s	V per CH_2 Å ³
Trilaurin, β -form Vand and Bell (45)	4.287	5.40	2.45	$74^\circ 45'$	$108^\circ 2'$	$117^\circ 24'$	23.7
<i>n</i> -Pentadecanoic acid A'-form (28)	4.25	5.82	2.61 ± 0.03	66°	106°	122°	25.0
<i>n</i> -Dodecanoic acid A-form (37)	4.42	5.41	2.63 ± 0.03	74°	109°	122°	25.0

every second chain-plane is almost perpendicular to the other planes. (Fig. 1 (a).) Vainshtein and Pinsker (44) have also determined the positions of the hydrogen atoms in the methylene groups. This information has been used when including the hydrogen atoms in the structure factor calculations.

The triclinic subcell has been fully described by Vand and Bell (45) in the case of trilaurin. It is probably the same as in a low temperature form of *n*-octadecane described by Müller and Lonsdale (46). It contains two CH_2 -groups and is shown in Fig. 1 (b). The dimensions of the subcell of trilaurin are collected in Table 2 together with those of the cells obtained from crystal forms studied in this investigation. In the triclinic packing of hydrocarbon chains all chain planes are parallel. When the subcells are distorted the values given in Tables 1 and 2 will be mean values.

It can be seen from Tables 1 and 2 that for the normal fatty acids the distance between alternate carbon atoms in a hydrocarbon chain (the c_s dimension) is longer in the triclinic packing than in the orthorhombic packing. All dimensions are

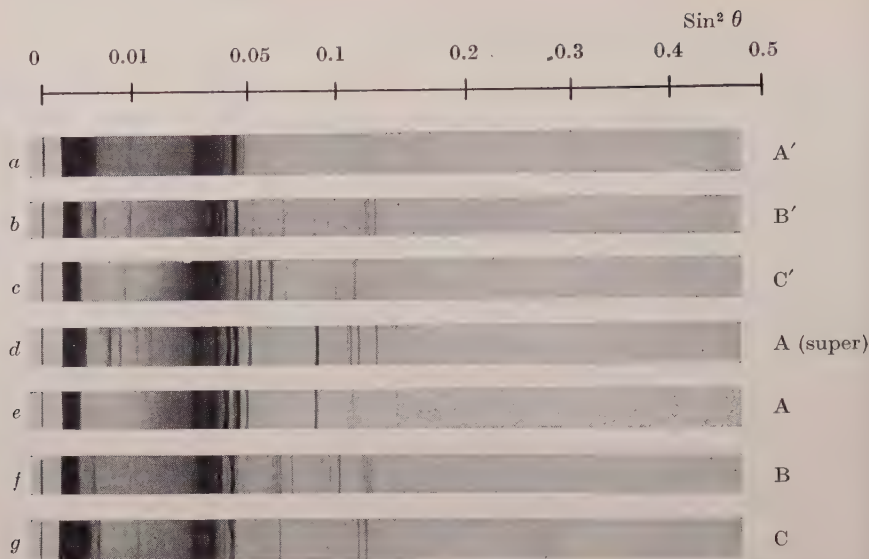


Fig. 2. Guinier powder photographs (Cu- $K\alpha$ radiation) of different crystal forms: (a) A' of *n*-pentadecanoic acid, (b) B' of *n*-heptadecanoic acid, (c) C' of *n*-hendecanoic acid, (d) A (super) of *n*-dodecanoic acid, (e) A of *n*-hexadecanoic acid, (f) B of *n*-hexadecanoic acid and (g) C of *n*-hexadecanoic acid.

dependent on the substituted groups and their positions and probably also on the chain length, even if the chains are packed principally in the same way. In the case of the orthorhombic packing the volume per CH_2 group is largest for the shortest acid and it decreases with increasing chain length down to the value for a long *n*-hydrocarbon.

Description of the crystal forms

As has been pointed out above there are three or four polymorphic forms of odd-numbered fatty acids and three of even-numbered acids. The D'-form has not been isolated nor has it ever been observed in the investigations carried out in Uppsala. Powder photographs of one member of each of the other crystal forms are shown in Fig. 2. The polymorphs will now be briefly described starting with those of the odd numbered acids.

Form A'

Form A' of *n*-pentadecanoic acid have been described in Refs. (28, 34). Its only symmetry element is a centre of symmetry and the cell is thus triclinic. The chains are packed roughly in accordance with the triclinic packing. The planes of the hydrocarbon chains are probably helically twisted in the case of *n*-pentadecanoic acid, but it can be supposed that the twisting will be less for longer members of this crystal form, i.e. the chains will approach the regular triclinic subcell with increasing chain length. This may imply that these long members have their chains strongly deformed near the carboxyl groups, but only there, to allow hydrogen bonding between two carboxyl groups. The hydrocarbon chains dominate the structure more

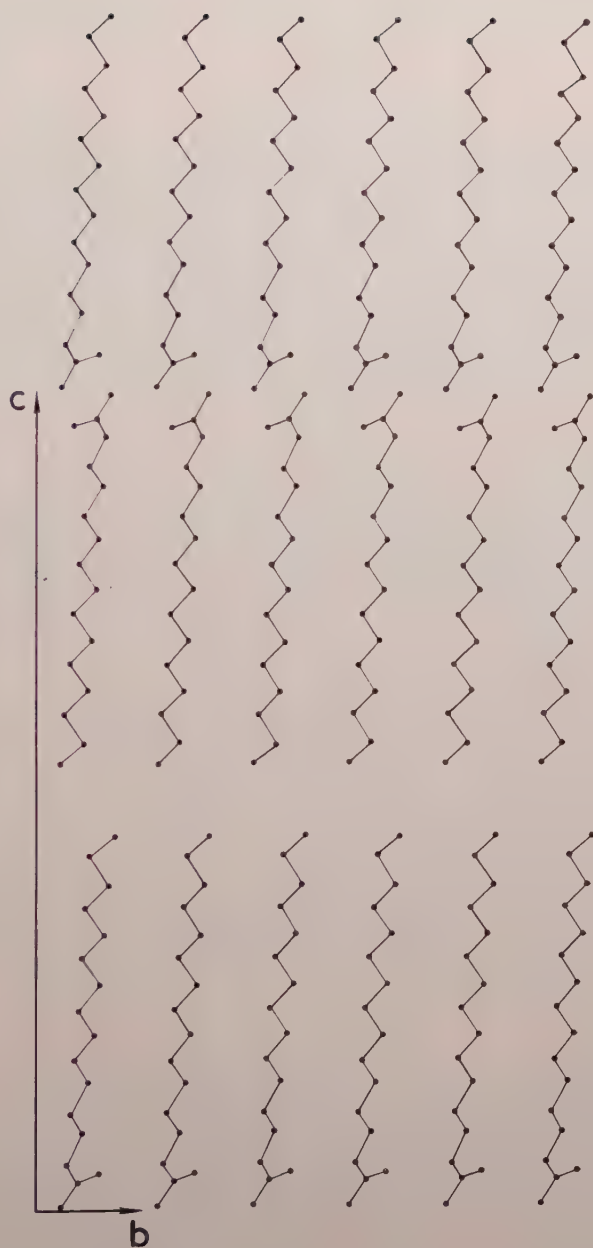


Fig. 3. The A'-form of *n*-pentadecanoic acid. Projection along the shortest axis on the largest axis plane.

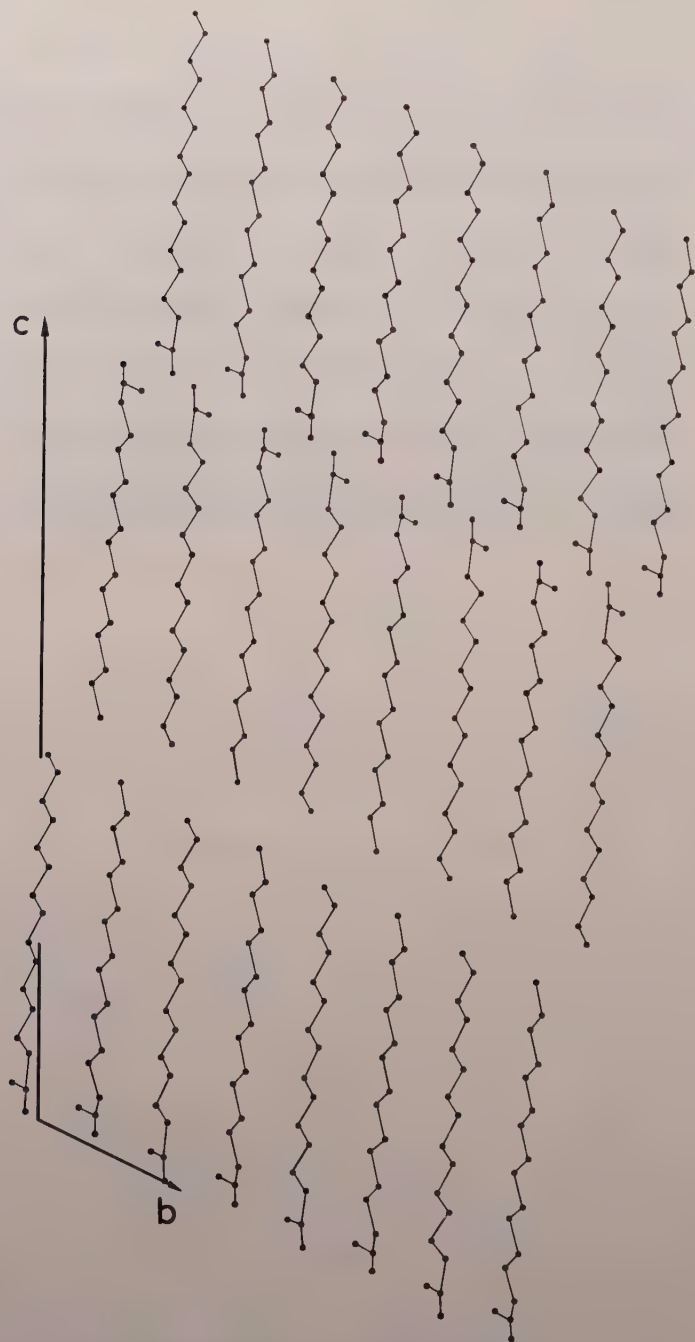


Fig. 4. The B'-form of *n*-pentadecanoic acid. Projection along the shortest axis on the largest axis plane.

and more, the carboxyl groups less and less, when the hydrocarbon chain in an acid molecule is lengthened. In Fig. 3 several unit cells are shown projected on the largest axis plane.

Form B'

Form B' of *n*-pentadecanoic acid (30) has the same symmetry as form A' but the hydrocarbon chains are orthorhombically packed. The packing is probably only deformed near the carboxyl groups, i.e. the hydrocarbon chains dominate the structure even at this comparatively short chain length. If the orthorhombic subcell is considered, the carboxyl groups are attached in the (111) plane of this subcell. (Compare with the above-mentioned suggestion made by Schoon (22).) In this case, however, the carbon atom in the carboxyl group had to be moved from its position in the subcell to allow hydrogen bonding between two fatty acid molecules. A projection along the shortest axis on the *bc*-plane is shown in Fig. 4.

Form C'

Form C' of *n*-hendecanoic acid (33) is monoclinic¹ with the chains arranged in accordance with the orthorhombic subcell. The carboxyl groups are situated in the (021)-plane of the subcell (if *a* is the shortest axis). Owing to the nearness of the melting point to the temperature of investigation the crystals used were very bad and always twinned. The intensity material was thus not so good. As can be supposed the coefficient B in the temperature factor $\exp. \left(-B \frac{\sin^2 \theta}{\lambda^2} \right)$ is extremely high: 9.7 Å². The temperature factor is not equal for every atom. In this structure it was very clearly seen in the difference syntheses that the thermal motion is larger in the methyl end of the molecule than in the carboxyl end which is held more tightly with hydrogen bonds.

In the case of *n*-hendecanoic acid the hydrocarbon chains of the C'-form are neither straight nor planar. This is probably caused by the effect of the comparatively large influence of the carboxyl group, due to the short chain, combined with the large thermal motion. If the chain is lengthened, the carbon atoms will certainly arrange themselves strictly in the orthorhombic subcell and the only deformation may be near the carboxyl group. A projected view of the actual crystal structure is shown in Fig. 5.

Form A

This crystal form behaves in a very peculiar way. It was first noted during a phase analysis that two X-ray powder photographs of *n*-dodecanoic acid, which were roughly equal and obviously both belonged to the A-form, differed in the low angle region. One had the usual appearance with evenly spaced long spacing reflexions (00*l*), but the other powder photograph showed a great number of unevenly spaced lines and no noticeable long spacing sequence.

It was possible to obtain a single crystal of the latter kind and its crystal structure has been determined (37). It is triclinic with the chains arranged strictly in the triclinic

¹ The *a*-axis has inadvertently been given a wrong value in (33). It should be 9.822 Å.

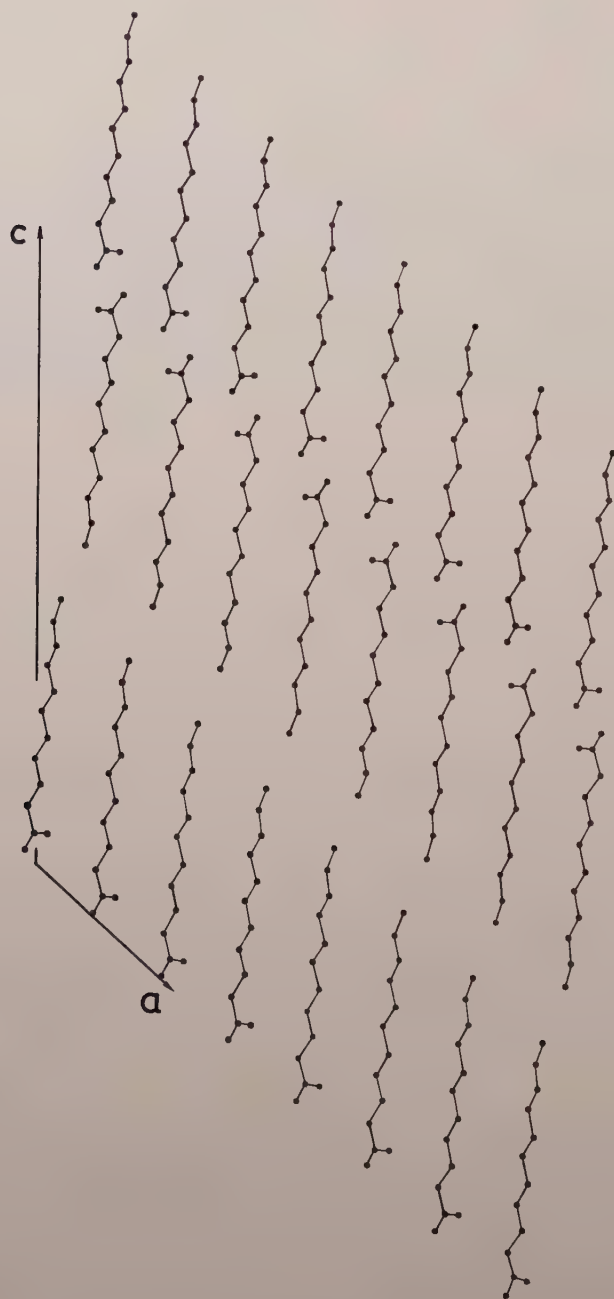


Fig. 5. The C'-form of *n*-hendecanoic acid. Projection along the shortest axis on the largest axis plane.

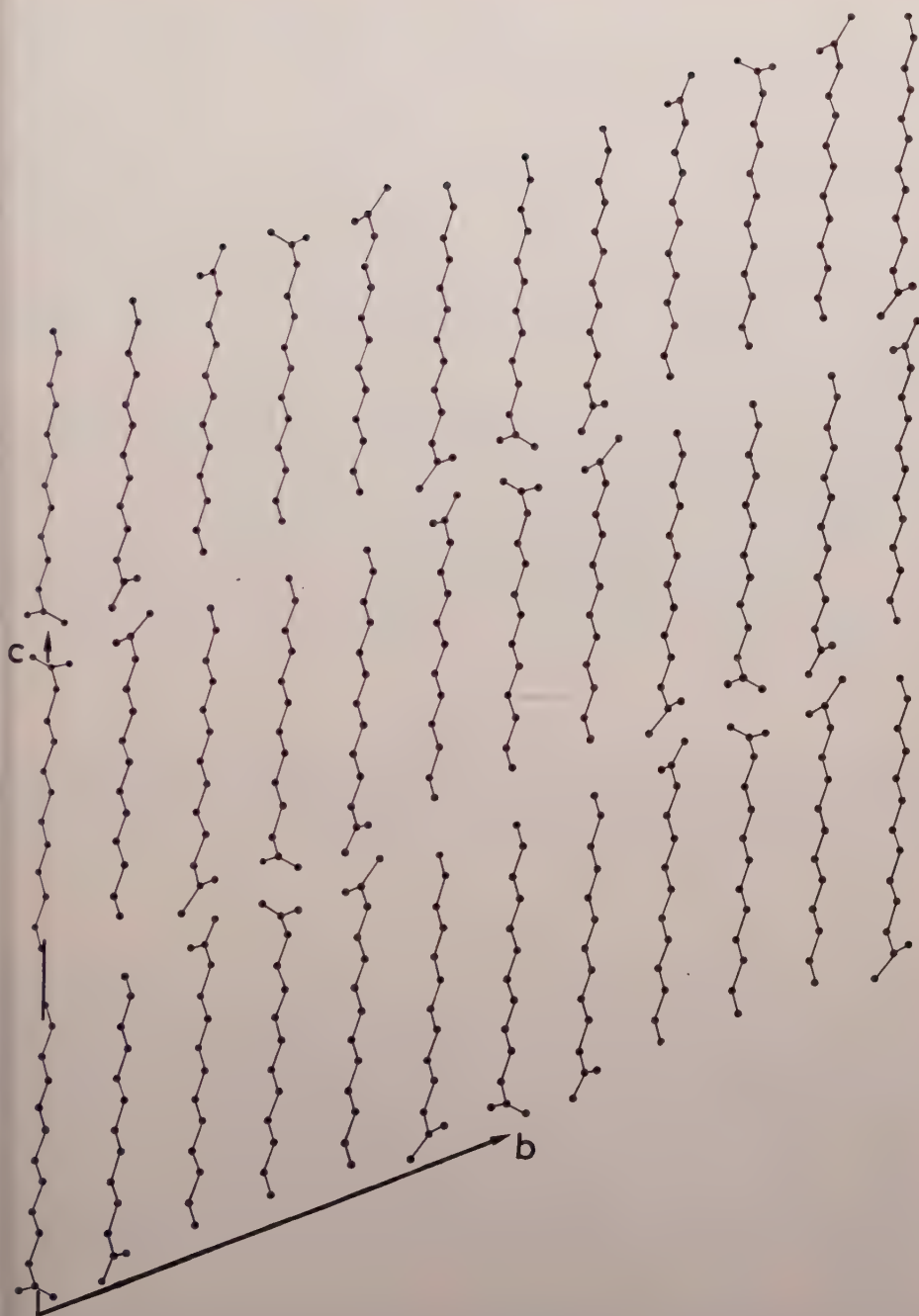


fig. 6. The A-form (superstructure) of *n*-dodecanoic (lauric) acid. Projection along the shortest axis on the largest axis plane.

manner. There are six molecules in the primitive unit cell, but by choosing the cell in the common way, i.e. with the smallest axis-plane parallel with the carboxyl group planes, there will be twelve molecules per unit cell (A-centered), and the structure can be regarded as a sixfold superstructure from the chainpacking point of view (Fig. 6).

The dimers are arranged so that there appear carboxyl groups and methyl groups in the same planes in the crystal. This causes the ordinarily strong, odd long spacing reflexions to disappear.

It is very striking that the subcell and the centered maincell have almost identical angles and orientations and that the actual dimensions are almost equal, cf. (37). With this arrangement of the molecules the carboxyl groups "disappear" in the packing of the hydrocarbon chains and from a crystallographic point of view the crystal is very like that of the low-temperature form of *n*-octadecane, investigated by Müller and Lonsdale (46).

This peculiar arrangement has only been found for *n*-dodecanoic and *n*-tetradecanoic acid (*n*-decanoic acid has not been investigated). The arrangement without superstructure has been found for these acids as well as for other even-numbered acids, but it has not been possible to obtain any single crystals of sufficient size. From what has been said about the powder photographs of the two arrangements, one can rather certainly conclude that in the other arrangement the dimers are arranged as in the other crystal forms with carboxyl groups and methyl groups in alternate layers but that the main cell and the chain packing still are triclinic. The probable number of molecules in the cell will be two. This structure resembles the A'-structure.

Form B

The B-form of *n*-octadecanoic (stearic) acid (31) is monoclinic with the orthorhombic packing and the carboxyl groups in the (101) plane of the subcell. The carbon atom in the carboxyl group is forced from its place in the subcell to allow the dimerization. A projection on the largest axisplane is shown in Fig. 7.

Form C

Vand, Morley and Lomer (26) determined the crystal structure of the C-form of *n*-dodecanoic (lauric) acid. It closely resembles the C'-structure. It has monoclinic symmetry, orthorhombic packing of the hydrocarbon chains and the carboxyl groups in the (021)-plane of the subcell (if *a* is the shortest axis). In the short axis projection the chains seem to be regular but in the bounded projection carried out along the medium axis the chains are clearly bent. This is very peculiar as the chain planes are neither perpendicular to nor parallel with any of the mentioned axes. Anyhow it can be assumed that for the longer members of this crystal form the carbon atoms are situated strictly in the orthorhombic subcell, except possibly the carbon atom in the carboxyl group. A projection along the shortest axis is shown in Fig. 8.

In Table 3 the structures of the different crystal forms are summarized. The angles of tilt given in (28, 30, 31, 33, 37) have been recalculated using the c_s values in Tables 1 and 2.

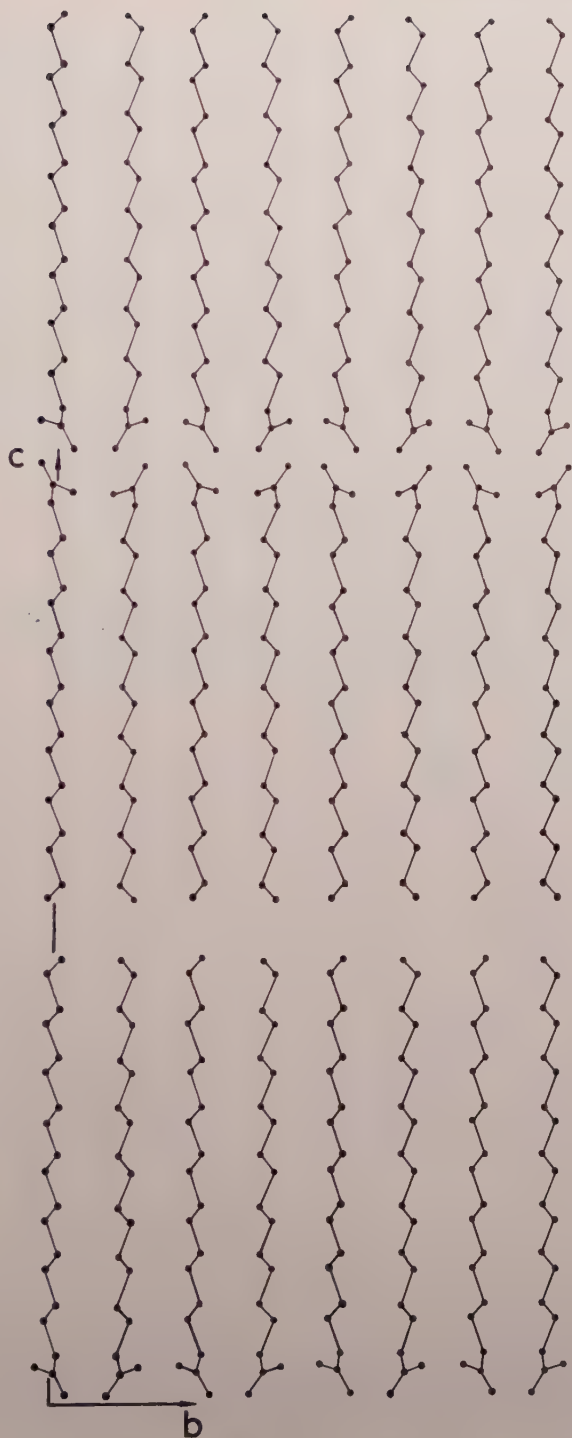


Fig. 7. The B-form of *n*-octadecanoic (stearic) acid. Projection along the shortest axis on the largest axis plane.

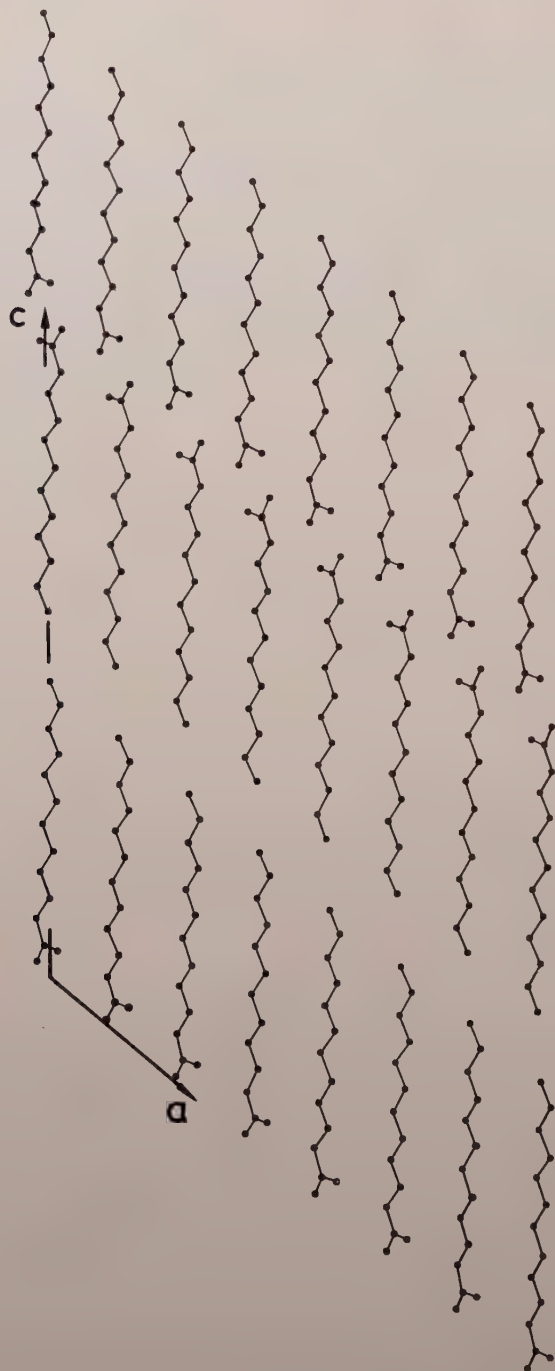


Fig. 8. The C-form of *n*-dodecanoic (lauric) acid. Projection along the shortest axis on the largest axis plane.

Table 3. Survey of the crystal forms of normal fatty acids.

Crystal form	Number of carbon atoms in the investigated acid	Space group (if shortest axis is a)	Sub-cell ¹	Subcell plane with carboxyl groups (if shortest axis is a)	Number of molecules in the unit cell	c_s Å	$\Delta d(001)$ per carbon atom Å (27)	Angle of tilt
A'	15	$P\bar{1}$	T		2	2.61	2.39	66°
B'	15	$P\bar{1}$	O	(111)	4	2.51	2.20	61°
C'	11	$P2_1/b$	O	(021)	4	2.52	2.15	59°
A	12	$A\bar{1}(P\bar{1})^2$	T		12(2) ²	2.63	2.36	64°
B	18	$P2_1/a$	O	(101)	4	2.53	2.32	66°
C	12	$P2_1/b$	O	(021)	4	2.52	2.09	56°

¹ T = triclinic subcell.

O = orthorhombic subcell.

² The values in brackets are for the other possible arrangement (see text).

Variation of unit cell dimensions

In the case of the C-form of even-numbered acids the unit cell dimensions of several fatty acids have been determined using the Guinier powder method (29). It was found that all dimensions of the monoclinic unit cell varied with the chain length. a , b , and β decreased asymptotically and c and $d(001)$ increased linearly with increasing chain length. Thus from a structural point of view the series is not strictly homologous.

In Fig. 9 the densities, calculated with the data in (29) for the different members have been plotted against the number of carbon atoms. The densities of the even-numbered acids form a smooth curve, which has a maximum at about 18 carbon atoms (n -octadecanoic acid). There are at least two factors influencing the variation in the densities: increase of low density material (CH_2 groups), which should cause a monotone decreasing density function, and denser packing of the molecules, due to increased total van der Waals interaction between the chains, which should cause an increase in the density. The second effect, which dominates up to 18 or 20 carbon atoms, changes the orientation of the chains. In Fig. 8 it can be seen that in the case of n -dodecanoic acid the chains form a small angle with the c -axis. As the hydrocarbon chains dominate the structure more with increasing chain length, the chain direction and the c -axis will become parallel. Considering this and the fact that the increment in the c -axis is constant it means that the c_s -dimensions, i.e. the distance between alternate carbon atoms, decrease somewhat with increasing chain length, probably asymptotically.

Physical properties of the crystals

The preparation of single crystals suitable for X-ray work has sometimes been very difficult. Either the crystals were too small or if the size was sufficient they were twinned or bent. The difficulties were particularly great in the case of odd-numbered acids. Crystals of normal fatty acids usually grow in thin plates. This is in accordance with their crystal structures. The molecules are arranged in sheets

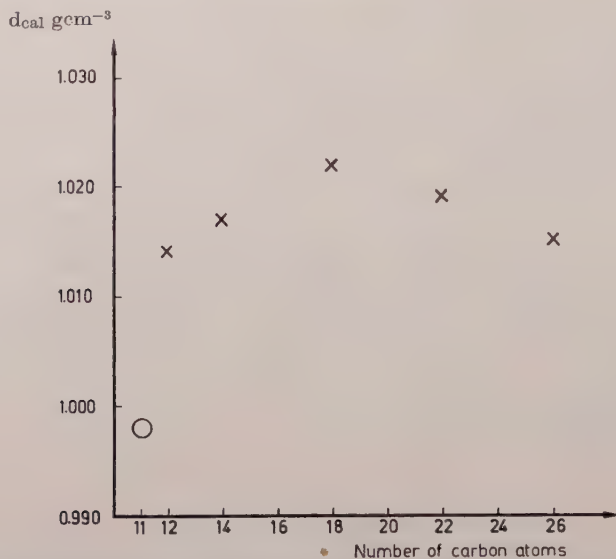


Fig. 9. The densities of the C-forms of several even-numbered fatty acids and of the C'-form of *n*-hendecanoic acid plotted against the number of carbon atoms.

within which the van der Waals interaction is strong owing to the closepacking of the hydrocarbon chains. These sheets are held together with hydrogen bonds and weak van der Waals bonds in alternate layers, i.e. the carboxyl group layers and the methyl group layers, respectively (Figs. 3-5, 7, 8), which will cause the crystals to have well-developed (001)-faces. In two cases, the superstructures of form A of *n*-dodecanoic and *n*-tetradecanoic acid, the crystals were not thin plates, but thicker parallelepipeds. This is easily explained, as in the superstructure the carboxyl and the methyl groups appear in the same layers and there are no layers with only weak van der Waals bonds (Fig. 6).

The cleavage is also consistent with these arrangements. The crystals, except those with superstructure, are easily split up in the (001)-planes.

Among the four crystal forms with orthorhombic chain packing (B', C', B, C) twinning is very common and in all observed cases the twins always have their (001)-planes parallel. This is also consistent with the weak van der Waals interaction in the methyl group layers.

No twinning has been observed in the A'- and A-forms, which have triclinic chain packing.

In the case of the B'-, B- and C-forms the crystal plates sometimes have well developed side faces which can easily be identified as (110) and ($\bar{1}\bar{1}$ 0). In Fig. 1 (a) it can be seen that these planes almost coincide with the planes of the hydrocarbon chains.

The crystals of all polymorphs are biaxial positive. As the chain direction is parallel with the acute bisectrix, it was very useful to know the direction of this bisectrix in the crystal when mounting it for X-ray work.

Infrared absorption

Sinclair, McKay and Jones (47) and Bellamy (48) found that the B- and C-forms of *n*-octadecanoic acid have different infrared absorption especially near $11\ \mu$, and Jones *et al.* (49) noticed that the number and positions of the evenly spaced absorption peaks near $8\ \mu$ (band progression) are dependent on the chain length. The author (32) has determined the infrared absorption between 5 and $13\ \mu$ for the different crystal forms of a few fatty acids, which were investigated as mulls in nujol, and for the melts of two acids. It was found that it is possible to distinguish between the different polymorphs, but that the chain length also must be considered.

The exact origins of the different absorption peaks are uncertain, especially at higher wavelengths, but by comparing the effect of changing crystal form and chainlength of several related compounds with known crystal structures it might be possible to explain the physical cause of the different absorption peaks.

The peak near $5.9\ \mu$, was found to be double as predicted by Davies and Sutherland (50), who attributed it to the C—O stretching vibration in dimers. Jones *et al.* (49) have attributed the band progression near $8\ \mu$ to wagging and twisting of the methylene groups in the hydrocarbon chains and Hadži and Sheppard (51) consider the absorption near $11\ \mu$ to be due to the OH-out-of-plane deformation. The last absorption region is, however, very complex and the exact origin highly uncertain.

Formation and existence of the polymorphs

As mentioned above the formation and existence of the different crystal forms are dependent on the temperature, the purity of the acid and the mode of crystallization, i.e. rate of crystallization and solvent used. The solvent can be supposed to affect the crystallization in at least two ways. A solvent with high vapor pressure vaporizes quicker than one with lower vapor pressure and thus the rate of crystallization will be larger. There is also an interaction between the polar fatty acid molecules and the solvent molecules, the interaction being stronger in polar solvents than in non-polar. The existence of these van der Waals forces (dipole-dipole or dipole-induced dipole) must be supposed to influence the formation of the crystalline solid. It is also very probable that the rate of formation is different for the different crystal forms. If one phase has a higher rate of formation than another, it can be formed by rapid crystallization, even if it is less stable.

It is of course much simpler to study the effect of crystallization rate exclusively, than it is to study the above-mentioned solvent effect exclusively, as it is easy to vary the crystallization rate using one solvent, but difficult to change solvent keeping the rate of crystallization constant. In Refs. (27, 36) these matters have been studied by crystallization of the fatty acids from polar and non-polar solvents at different temperatures. The transition points for the different phases (27) have been determined in a continuously recording temperature camera designed by Stenhagen (52). The odd- and even-numbered fatty acid series behave entirely differently, so they will be discussed separately.

In no case have any solvates been detected.

Odd-numbered fatty acids

The B'-form of odd-numbered acids is the most common at room temperature and has been considered to be the most stable one. Crystallization at room temperature

from solution and from melt always give B' in the case of *n*-heptadecanoic acid and longer acids, but by lowering the temperature, i.e. the rate of crystallization, the A'-form is obtained in some cases for the first mentioned acid. With decreasing chain length the A'-form becomes more common and it is the only one found for *n*-tridecanoic acid. de Boer (8) found A' and B' together for this acid, but this is probably an effect caused by impurities. A similar result was obtained by the author with an impure specimen of *n*-heneicosanoic acid, which crystallized from light petroleum in A' and B'. With a pure specimen of this acid or the neighbouring acids it was impossible to obtain the A'-form by crystallization at room temperature. When an A'-form is heated, it is transformed to B' (27), which at higher temperatures transforms to C' but in the case of *n*-tridecanoic acid, A' transforms directly to C'. In fact the extrapolated transition point curve A'→B' (complemented with the point at 59°C for *n*-tricosanoic acid given by Kohlhaas and Stüber (25)), crosses the B'→C' curve a little under 15 carbon atoms and the melting point curve a little above 13 carbon atoms, which makes the existence of the B'-form of pure *n*-tridecanoic acid highly improbable.

When the B'-form of *n*-nonadecanoic acid was kept at 45°C for two hours (27) it transformed completely into the A'-form. This together with the mentioned fact, that in the case of *n*-heptadecanoic acid (36) lowering of the speed of crystallization gave A'-phase instead of B'-phase, means that the stable crystal form under the A'→B' curve (27) is A' and not B', but that the rate of formation is slower for A' than for B'. The rate of transition, B'→A' in the case of *n*-nonadecanoic acid is obviously very low at room temperature, but it rises of course with temperature and at 45°C the transition takes place in less than two hours. This may be explained if one recalls that the stable room-temperature forms of hydrocarbons are those with the orthorhombic chain packing (53, 54) with the chains arranged as in the B'-form of odd-numbered fatty acids. With a medium long chain in the acid molecules the rate of formation is highest for the case with the chains arranged orthorhombically (B') but the total van der Waals interaction is larger in the A' structure with triclinic chain packing. With a short chain (less than 15 carbon atoms) not even the rate of formation is dominated by the hydrocarbon chains, but with a sufficiently long chain both rate of formation and crystal structure can be supposed to be dominated by the chain, and the stable crystal form will probably be B'.

Just below the melting point of the acid the only possible crystal form is C' (27) which means that the solid phase in equilibrium with liquid phase is always of the C'-form.

The dipole effect of the solvent is difficult to study as the rate of crystallization is not so easily controlled. *n*-Pentadecanoic acid, which mostly crystallizes in the A'-form (36), gave B' together with A' from acetone at room temperature, and pure B' when crystallized from glacial acetic acid and from melt (27). These solvent molecules are the strongest dipoles used in this investigation, and the second one has a low vapor pressure (m.p. 17°C), i.e. the rate of crystallization is very low, which properly favours formation of A'-phase. In the case of acetone, which has a high vapour pressure at room temperature, it is hard to say what causes the formation of B' together with A'. However, if one compares crystallization from acetone with that from pentane (36), which also has a high vapor pressure at room temperature but is non-polar, it seems that a strong dipole moment favours formation of the metastable B'-phase, which has the chains packed in the orthorhombic manner.

Slagle and Ott (10) suggest that the D'-form of odd-numbered fatty acids, first noticed by Piper (55), only exists in impure materials. The author has never observed the existence of a D'-phase in his investigations.

Even-numbered fatty acids

It is much more difficult to make any general predictions about the formation of the polymorphic forms of the even-numbered fatty acids than of the odd-numbered. Crystallization from melt and rapid crystallization from solution at room temperature always give the C-form, which means that this form has the highest rate of formation for all chain lengths investigated. This form has also been considered to be the stable one. Crystallization from solution (27, 36) can give A, B, and C, singly or two together, but very seldom A and B together. Slow crystallization (27, 36) favours formation of A or B except when the solvent molecules are strong dipoles, e.g. glacial acetic acid which seems to cause formation of C singly or together with A or B. The B-form has never been found for *n*-dodecanoic acid or *n*-tetradecanoic acid, but for all other acids investigated it has appeared (27, 36). The A-form can exist for all acids up to and including *n*-docosanoic (behenic) acid. This agrees well with the transition point curves $A \rightarrow C$ and $B \rightarrow C$, which cross each other at about 24 carbon atoms (27).

There are many experimental results which suggest that A and B are more stable than C and that at shorter chain lengths (less than 24 carbon atoms) A is more stable than B. As was mentioned above slow crystallization from solutions in non-polar solvents favours formation of A and B. The B-forms apparently do not exist for *n*-dodecanoic and *n*-tetradecanoic acid. Lomer (15) found that grinding and preservation of the C-form of *n*-dodecanoic or *n*-tetradecanoic acid gave the A-form. From the transition point curves (27) it can be seen that up to 24 carbon atoms more energy is required to transform A to C than to transform B to C for the same acid. Thus it is almost certain that the A-form with triclinic chain packing is the stable one up to about 22 carbon atoms and that the B-form with orthorhombic chain packing is stable for the longer members of this series investigated. The analogy with the odd-numbered acids is striking. In this case, however, the hydrocarbon chains dominate the rate of crystallization for all members, even the shortest. As a comparison it can be mentioned that the cell dimensions of the C-form of even-numbered fatty acids (29) assume the asymptotic values at about 24 carbon atoms.

Of the two possible arrangements of the A-forms of *n*-dodecanoic and *n*-tetradecanoic acid the superstructures are the most common and probably the most stable of the two variants. For *n*-dodecanoic acid the transition point A (super.) $\rightarrow$ C is 3° higher (35°C) than the $A \rightarrow C$ transition, which strengthens this suggestion.

Solid acid in equilibrium with melt is always of the C-form for the acids investigated.

Alternation in the melting points

In 1877 Baeyer (56) found that the melting points of the normal fatty acids and the dibasic normal acids, plotted against the number of carbon atoms describe an alternating, rising curve, the odd-numbered fatty acids having comparatively lower melting points. Biach (57) extended this concept to comprise all long-chain compounds and other physical properties, e.g. boiling points and solubility. This was, however, later found to be wrong and the present state of knowledge is that the alternation

is connected with properties of the solid state only and with certain homologous series, e.g. the normal fatty acids and the methyl esters of normal fatty acids, but not the normal alcohols (at least up to 29 carbon atoms, Stållberg *et al.* (39)) and the paraffins. It is well known that the alternation becomes less pronounced when the chain length is increased. Malkin (58, 59) discussed the phenomena considering the methyl group packing and put forward the hypothesis, that non-alternating series possess vertical chains while alternating series possess tilted chains.

Considering the above mentioned properties there is probably no difference in molecular structure. Thus, there must be a difference in the packing of the molecules in the solid state and the van der Waals interaction between them. The hydrogen bonding between two molecules need not be discussed as the acids certainly exist as dimers even in the liquid state at least in equilibrium with solid phase, i.e. at the melting point. If the series were homologous even from the crystallographic point of view, i.e. the cell-dimensions could be evaluated from continuous functions of the number of carbon atoms, the only difference between the odd and even members should be the regular increment in van der Waals interaction between side-packed molecules caused by the lengthening of the hydrocarbon chains. This is probably the case with the alcohols and the paraffins.

It has previously been stated that the only crystal forms which can be in equilibrium with liquid phase are the C-form of even fatty acids and the C'-form of odd acids. These two crystal forms have the same orthorhombic chain packing, the same space group and the carboxyl groups situated in the same plane in the orthorhombic subcell and must thus differ in some minor details, at least when the chain is not too long. In Fig. 9 the densities of the C-forms of acids with 12, 14, 18, 22 and 26 carbon atoms, calculated from the data given in (29) and discussed earlier in this paper, are plotted against the number of carbon atoms together with the density of the C'-form of *n*-hendecanoic acid calculated from the corrected cell-data. It can be seen clearly that the density of the C'-form in question is appreciably lower than the densities of the C-forms, even within the limits of experimental error. This indicates that the molecules of the C'-form are less tightly packed than those of the C-form.

When a normal fatty acid crystallizes from melt the molecules arrange themselves parallel to each other in a way which is the same for odd- and even-numbered acids (compare the observation by Stenhagen (60) that there does not seem to be any alternation in the properties of compressed monolayer surface films). These sheets, which are roughly $d(001)$ thick, are however, owing to the zig-zag shape of the chain bounded in different ways in the two cases. For the even-numbered acids the last covalent carbon-carbon bond in a chain is very roughly parallel with the carboxyl group plane but for the odd-numbered acids the bond in question is roughly perpendicular to this plane (compare Fig. 5 with Fig. 8). This causes the sheets to arrange themselves in different ways for the two cases, seeking the largest possible van der Waals interaction.

The contact regions of interest are shown in enlargement in Fig. 10. Assuming the ideal orthorhombic packing described by Bunn (43) and Vainshtein and Pinsker (44) distances less than 5.5 Å between the carbon atom in one methyl group and all possible carbon atoms on the opposite side of the methyl gap have been calculated and collected in Table 4 for the two cases.

The van der Waals interaction between simple molecules is dominated by the dipole-dipole term, which is conventionally written in the form $-nR^{-6}$, London (61). In order to get a rough estimate of the order of magnitude of the difference between

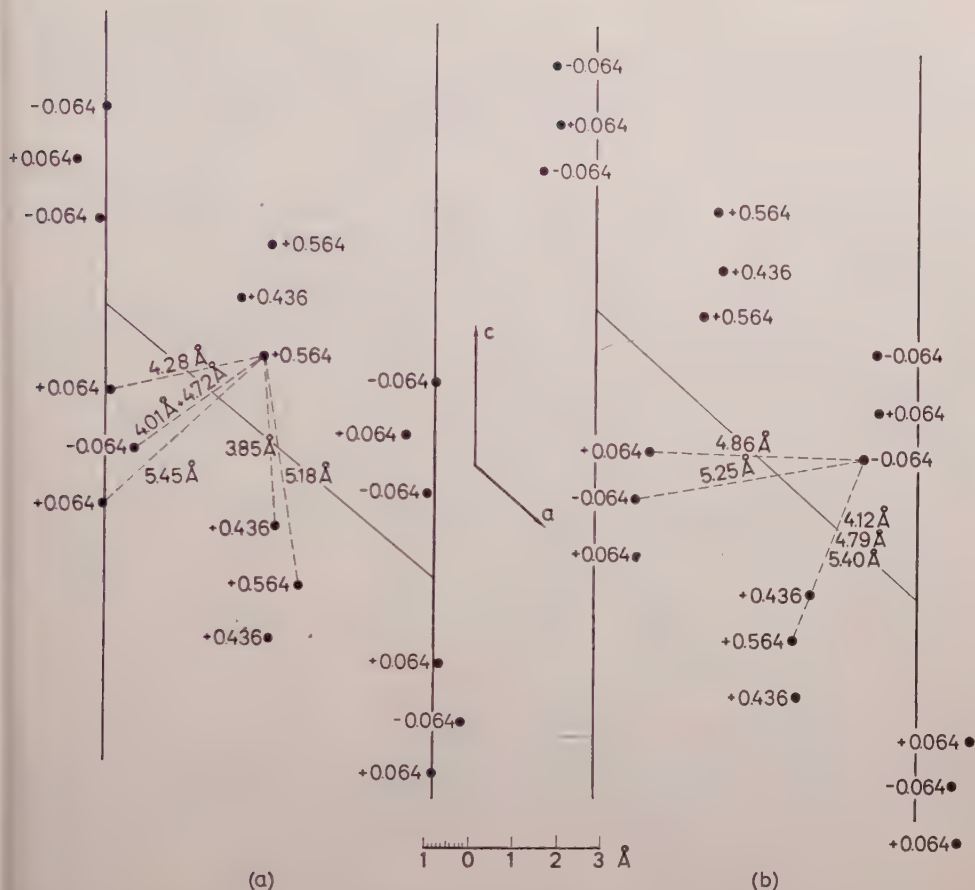


Fig. 10. The methyl-group regions of (a) the C-form of *n*-dodecanoic (lauric) acid and (b) the C'-form of *n*-hendecanoic acid. *y*-coordinates from Bunn (43) and Vainshtein and Pinsker (44).

the van der Waals interaction in the two cases, the interactions between the carbon atom in one methyl group and all carbon atoms on the other side of the gap (closer than 5.5 Å) have been calculated with the formula mentioned. The influence of the hydrogen atoms has been ignored. The values obtained are collected in Table 4 and are given on an arbitrary energy scale. It can be seen that with the given conditions the interaction is more than 60 % larger in the C-form of *n*-dodecanoic acid than in the C'-form of *n*-hendecanoic acid. This is in accordance with the comparatively low melting point and heat of crystallization (Garner *et al.*, 62) of *n*-hendecanoic acid. This effect might also partly be caused by less dense packing of the hydrocarbon chains accompanied by lower van der Waals interaction between side-packed molecules in the cases of the C'-forms of odd-numbered fatty acids, Tables 1 and 2. The alternation becomes less pronounced as the chains, with increasing chain length, become parallel with the *c*-axis, and the carboxyl groups lose their influence on the packing. Fatty acids with sufficiently long hydrocarbon chains will thus behave as paraffins and will not show any alternation in their properties.

For comparison, the van der Waals interaction between one carbon atom within a hydrocarbon chain and all carbon atoms (closer than 5.5 Å) in adjacent molecules, arranged in the orthorhombic manner, have been calculated under the same conditions. The values, which are on the same energy scale, are given in Table 5.

Table 4. Distances R , ($R < 5.5$ Å) and simplified van der Waals interactions, E , (see text) between the carbon atom in one methyl group and all possible carbon atoms on the other side of the methyl gap in the C-form of lauric acid and the C'-form of n -hendecanoic acid (see Fig. 10).

The C-form of lauric acid			The C'form of n -hendecanoic acid		
Carbon-carbon distances R Å	Number of distances n	$-E = nR^{-6} \times 10^4$	Carbon-carbon distances R Å	Number of distances n	$-E = nR^{-6} \times 10^4$
3.85	1	3.1	4.12	2	4.1
4.01	1	2.4	4.79	1	0.8
4.28	2	3.2	4.86	1	0.8
4.72	1	0.9	5.25	1	0.5
5.18	1	0.5	5.40	1	0.4
5.45	2	0.8			
$\Sigma nR^{-6} \times 10^4$		10.9	$\Sigma nR^{-6} \times 10^4$		6.6

Table 5. Distances, R , ($R < 5.5$ Å) and simplified van der Waals interaction, E , (see text) between a carbon atom in a hydrocarbon chain and all possible carbon atoms in adjacent molecules arranged in the common orthorhombic way (Fig. 1 (a)).

Carbon-carbon distances R Å	Number of distances n	$-E = nR^{-6} \times 10^4$
4.13	2	4.0
4.20	4	7.3
4.83	6 (4 + 2)	4.7
4.94	2	1.4
5.09	4	2.3
$\Sigma nR^{-6} \times 10^4$		19.7

Summary

Normal fatty acids exhibit polymorphism. A review of earlier crystal structure investigations is given.

The structures of the six different crystal forms are described. The hydrocarbon chains are found to be packed in two ways, orthorhombic and triclinic.

The infrared absorption of the different crystal forms has been determined.

The formation and existence of the polymorphs are discussed. The formation is dependent on the temperature, the solvent used, the rate of crystallization and on

impurities. With reasonably short chain length the stable forms have triclinic chain packing but with longer chains (probably about 23 carbon atoms and more) the stable forms have orthorhombic chain packing.

The well-known alternation in physical properties of the solid state is discussed and correlated with the actual crystal structures determined.

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On the reactions between dithiocarbhydrazides and formaldehyde

By JAN SANDSTRÖM

With 2 figures in the text

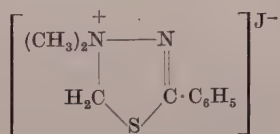
I. Introduction and survey

THIELE and BAILEY (1) and MICHAEL (2) have reported that semicarbazide reacts with formaldehyde in acid solution in the molar proportion 2:3, but the structure of the resulting product has not been settled. The reactions between phenylhydrazine and formaldehyde have been investigated by, among others, WELLINGTON and TOLLENS (3), GOLDSCHMIDT (4), and WALKER (5). WELLINGTON and TOLLENS obtained a compound, also resulting from two moles of phenylhydrazine and three moles of formaldehyde, and GOLDSCHMIDT described an isomer of this compound. In neither case has the structure been settled. WALKER described a monomeric and a dimeric formaldehyde phenylhydrazone and an oxygen-containing compound resulting from the reaction between two moles of phenylhydrazine and four moles of formaldehyde.

HOLMBERG (6, 7) has investigated the reactions between thiobenzhydrazide and formaldehyde. He describes a formaldehyde thiobenzhydrazone, a compound resulting from reaction between two moles of thiobenzhydrazide and three moles of formaldehyde, methylene-bis-methylenethiobenzhydrazide, and a compound resulting from the reaction between one mole of thiobenzhydrazide and two moles of formaldehyde in alkaline medium, methylene-hydroxymethylthiobenzhydrazide. The formaldehyde thiobenzhydrazone and the methylene-hydroxymethylthiobenzhydrazide are labile and are spontaneously transformed into the methylene-bis-methylenethiobenzhydrazide. All three compounds gave the same methiodide when they reacted with methyl iodide. Since the same methiodide was formed when the 2-methylthiobenzhydrazide (I) reacted first with formaldehyde and then with methyl iodide, the methiodide must be 2-phenyl-4,4-dimethyl- Δ^2 -1,3,4-thiadiazolinium iodide (II).

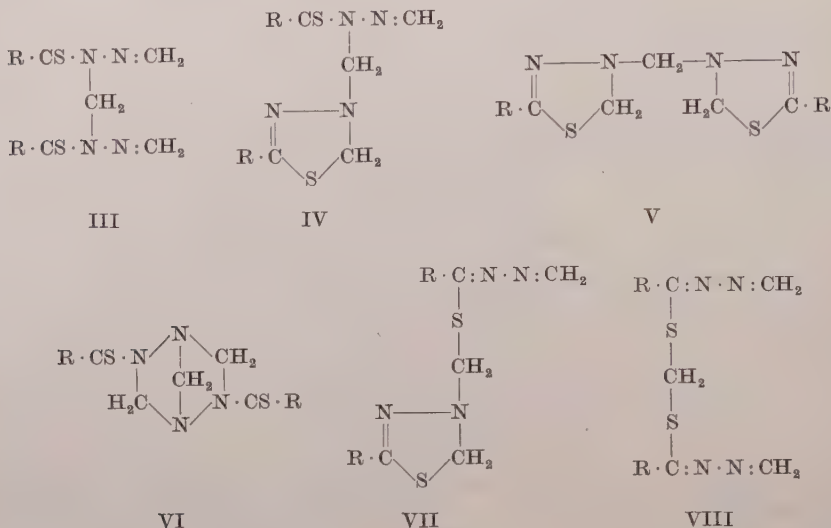


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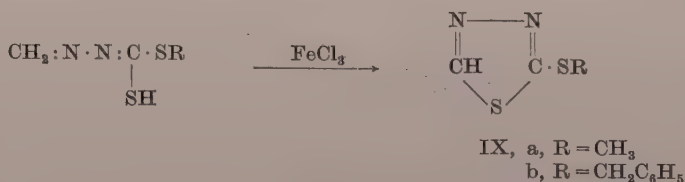
II

For the methylene-bis-methylenethiobenzhydrazide several structures are possible. The following six can be more seriously considered ($R = C_6H_5$):

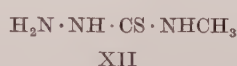
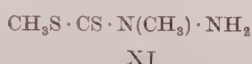
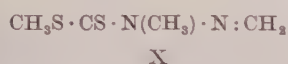


The unsymmetrical structures (IV) and (VII) have been taken into consideration, because when methylene-bis-methylenethiobenzhydrazide reacted with thioglycollic acid, a crystalline compound, resulting from the addition of only one mole of thioglycollic acid and consequent anhydridisation, was isolated from the reaction products. HOLMBERG proposes the structure (III) for methylene-bis-methylenethiobenzhydrazide, though with reservation for other possibilities.

The dithiocarbhydrazides have been found to behave in many respects like thiobenzhydrazide in reactions with formaldehyde. When equimolecular amounts of dithiocarbhydrazides and formaldehyde reacted in alkaline solution the corresponding simple formaldehyde dithiocarbhydrazones resulted. They were non-crystalline, but like the formaldehyde thiobenzhydrazone they gave crystalline hydrochlorides. The methyl derivative has m.p. 116° – 117° and the benzyl derivative 115° – 117° . When the hydrochlorides were oxidized with iron (III) chloride, 2-methylthio-1,3,4-thiadiazole (IX a, liquid, hydrochloride m.p. 162° – 163°) and 2-benzylthio-1,3,4-thiadiazole (IX b) with m.p. 59° – 60° were obtained. The latter thiadiazole has recently been prepared by YOUNG and WOOD (8) from benzyldithiocarbhydrazide and formic acid. When formaldehyde thiobenzhydrazone was oxidized with iron (III) chloride, 2-phenyl-1,3,4-thiadiazole was obtained (9), and oxidation of methylene-bis-methylene thiobenzhydrazide with pyridinium tribromide gave the same product (7).

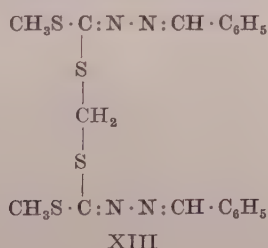


The free formaldehyde dithiocarbhydrazones are not stable but are transformed into compounds which are insoluble in alkali. Only from the methyl derivative was a crystalline product obtained, m.p. 47° – 48° (compound A). This compound was also formed, when two moles of methylthiocarbhydrazide reacted with three moles of formaldehyde in neutral medium. Compound A reacted with hydrazine hydrate to give thiocarbhydrazide in 66% yield. Elemental analysis and molecular weight determination gave the empirical formula $C_7H_{12}N_4S_4$. For this compound the structures (III–VIII, $R = CH_3S$) have been more seriously considered. Attempts have been made to prepare compounds with structures similar to (III–VIII) for chemical and spectroscopic comparison. For structure (III) formaldehyde 2-methyl-methyldithiocarbhydrazone (X), m.p. 65° – 66° , was used. The parent hydrazide (XI), m.p. 93° – 94° , was obtained by reaction between dimethyl trithiocarbonate and methyl hydrazine. The structure (XI) was demonstrated by reaction with nitrous acid, when the amino group was exchanged for hydrogen. This reaction



is common for 1,1-disubstituted hydrazines (10). The methyl methyldithiocarbamate thus formed is liquid and was characterized by reaction with hydrazine hydrate, which gave the previously known 4-methylthiosemicarbazide (XII, 11). The acetaldehyde and benzaldehyde hydrazones of (XI) were also prepared, m.p. 57° – 58° and 92° – 93° respectively. The infra-red and ultra-violet absorption spectra of (X), Diagram 1 and 2, are so different from those of compound A, that structure (III) seems very improbable for the latter compound.

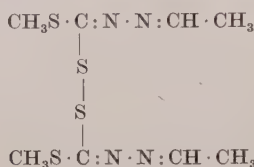
Attempts to prepare crystalline compounds with a structure similar to (VIII) by reacting methyldithiocarbhydrazones with methylene dihalides in alkaline solution have been successful only in one case, with benzaldehyde methyldithiocarbhydrazone, which gave dibenzaldehyde S'-methylene-bis-S"-methyl-isodithiocarbhydrazone (XIII), m.p. 130° – 131° . The presence of the phenyl nuclei gives the compound a very doubtful value for comparison with compound A. The compound (XIII) is



unaffected by hydrazine hydrate and by methyl iodide at room temperature, whereas compound A gives thiocarbhydrazide with hydrazine hydrate and a methiodide, which will be described later, with methyl iodide under the same conditions.

The acetaldehyde methyldithiocarbhydrazone, m.p. 92° – 93° , has not been described earlier. HULL claims this structure for a compound of the same composition with m.p. 166° – 167° (12) which was obtained when acetaldehyde and methyldithiocarbhydrazide reacted in aqueous ethanol at a slightly raised temperature. Possibly it

is a trimeride. The problem will be further investigated. When the hydrazone with m.p. 92°–93° was oxidized with iodine in alkaline solution the acetaldehyde methyl-isodithiocarbhydrazone disulphide (XIV) was obtained, m.p. 110°–111°. This structure has some similarity with structure (VIII). The infra-red absorption spectrum of the disulphide (XIV), Diagram 1, has a strong absorption at 6.12 μ which is also found in the spectrum of the acetaldehyde 2-methyl-methyldithiocarbhydrazone (XV). It can probably be assigned to the hydrazone $-N:CH-$ bond, since acetaldehyde



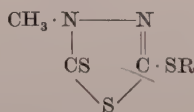
XIV



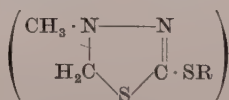
XV

azine is known (13) to absorb at 6.01 μ , which is ascribed to the $-N:CH-$ bond. The disulphide (XIV) has also a strong absorption band at 6.60 μ , which is tentatively assigned to the other >C:N- bond. The infra-red spectra of (XV) and of the corresponding formaldehyde hydrazone (X) between 5.4 and 7.2 μ , Diagram 1, differ mainly in the position of the absorption band in the region between 6.1 and 6.3 μ . The band of the formaldehyde hydrazone (X) occurs at 6.26 μ and is assigned to the $-N:CH_2$ bond. This absorption band is completely absent in the spectrum of compound A, and therefore the structures (III), (IV), (VII), and (VIII), which require this band are regarded as less probable.

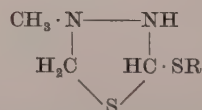
Attempts to prepare compounds with structures similar to (V) have not been successful. Attempts to hydrogenate 2-mercapto- or 2-methylthio-4-methyl-1,3,4-thiadiazole-5(4H)-thione (XVIa and b, 14) with sodium amalgam or with zinc and acetic acid to the corresponding Δ^2 -1,3,4-thiadiazolines (XVII) were in vain. The methyl and benzyl derivatives (XVIb and c) reacted readily with lithium aluminium hydride, but instead of the compounds (XVII) products with two more hydrogen atoms were obtained. These are tentatively regarded as 1,3,4-thiadiazolidines (XVIII),



XVI, a, R = H
b, R = CH₃
c, R = CH₂C₆H₅



XVII



XVIII, a, R = CH₃
b, R = CH₂C₆H₅

since azines are reduced by lithium aluminium hydride (15), but the light-absorption of the methyl derivative in the region 220–330 $m\mu$, Diagram 2, is far stronger than expected for a saturated compound.

However, the chemical properties of the reduction products, particularly the stability towards acid hydrolysis, are not compatible with any of the possible unsaturated structures. The compound (XVIIIa) has m.p. 95°–96° and (XVIIIb) 78°–79°. A low yield of (XVIIIa) was obtained when compound A was reduced with lithium aluminium hydride.

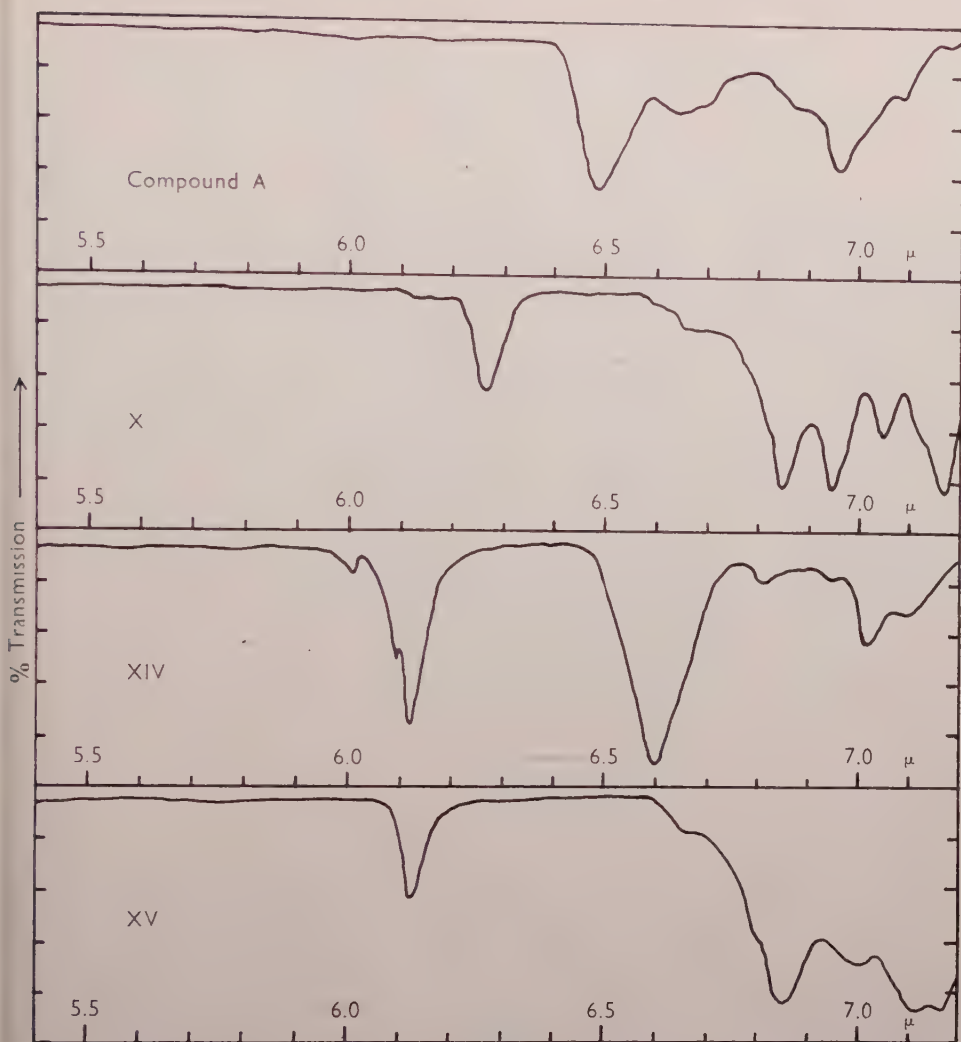


Diagram 1.

The infra-red absorption spectrum of compound A, Diagram 1, has a strong absorption band at 6.48 μ . OTTING and DRAWERT (16) have recently investigated a large number of thiazolines, which contain a $>\text{C}:\text{N}-$ bond similar to that in structure (V). They have correlated this bond with a strong absorption band in the region 6.1–6.45 μ . Obviously both chemical and infra-red absorption data are in agreement with the assignment of structure (V) to compound A, though the evidence is not absolutely conclusive.

Compounds with structures similar to (VI) have not been prepared. However, when compound A reacted with methyl iodide in ether or ethyl acetate solution, one methylene group was eliminated and a methiodide was formed, m.p. 173°–174°.

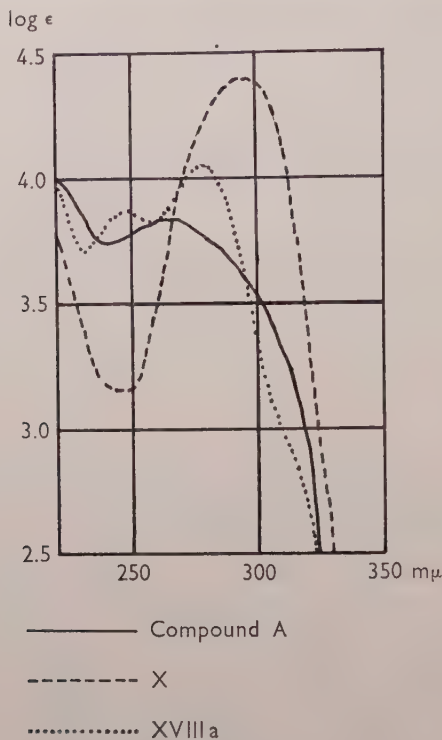
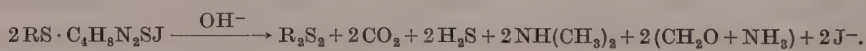


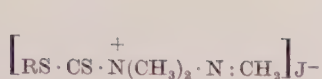
Diagram 2.

Elemental analysis and freezing-point depression of the water solution give the formula $C_5H_{11}N_2S_2J$ and not $C_{10}H_{22}N_4S_4J_2$. A methiodide of the latter formula would have been expected to result from structure (VI). When benzyldithiocarbhydrazide and formaldehyde reacted to give the benzyl analog of compound A, a non-crystalline product was obtained, which reacted with methyl iodide to give a methiodide, m.p. 159° – 160° , with a composition corresponding to that of the above-mentioned methyl derivative. Both were decomposed on heating in strongly alkaline solution with the formation of dimethylamine, carbon dioxide and hydrogen sulphide. From the benzyl derivative an almost quantitative yield of dibenzyl disulphide was also obtained. Formaldehyde and ammonia were also expected, but neither could be identified, probably due to secondary reactions. The decomposition obviously follows the formula:

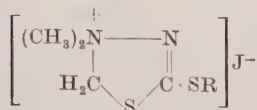


Since the two new methyl groups have become attached to the same nitrogen atom, only the two structures (XIX) and (XX) are possible for the methiodides.

As mentioned above, HOLMBERG (7) has proved the thiadiazolinium structure (II) to be correct for the corresponding thiobenzhydrazide derivative. It is also the most satisfactory for the dithiocarbhydrazide derivatives, since formaldehyde 2-



XIX

XX, a, R = CH₃
b, R = CH₂C₆H₅

methyl-methyldithiocarbhydrazone (X) does not react with methyl iodide, which it ought to have done if structure (XIX) had been correct for the methiodides. Neither structure (XX) nor the less probable (XIX) lends any support to structure (VI) for compound A, whereas structure (XX), if correct, gives a certain support to structure (V).

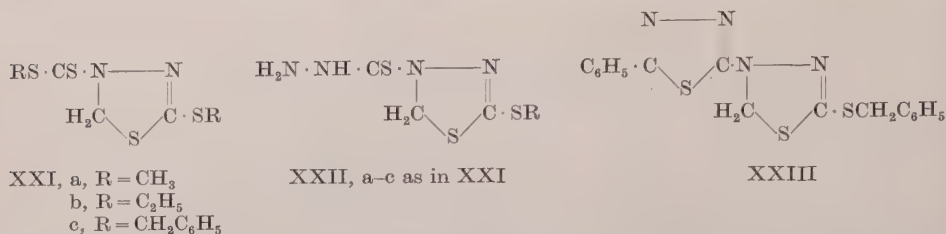
On the whole the available data tend to accumulate in favour of structure (V), and it is, therefore, tentatively proposed that compound A is bis-[2-methylthio- Δ^2 -1,3,4-thiadiazolin-4-yl]-methane.

No crystalline compounds corresponding to the methylene-hydroxymethylthio-benzhydrazide (7) have been found in the reactions between formaldehyde and dithiocarbhydrazides.

When two moles of methyldithiocarbhydrazide reacted with one mole of formaldehyde in acid solution, hydrazine hydrate was eliminated and a compound (XXIa) with the empirical formula C₅H₈N₂S₄ was formed, m.p. 143°–144°. The corresponding ethyl derivative (XXIb), m.p. 78°–79°, was obtained earlier (17), when the oil resulting from the reaction between ethyldithiocarbhydrazide and formaldehyde was oxidized with iodine or pyridinium perbromide. Probably the acid formed in the oxidation has effected the formation of the compound (XXIb). The corresponding benzyl derivative (XXIc) has m.p. 110°–111°. The methyl and benzyl derivatives were also obtained when the hydrochlorides of the simple formaldehyde dithiocarbhydrazones were heated in ethanolic solution, and when compound A was dissolved in acid solution a low yield of the above-mentioned methyl derivative (XXIa) was obtained.

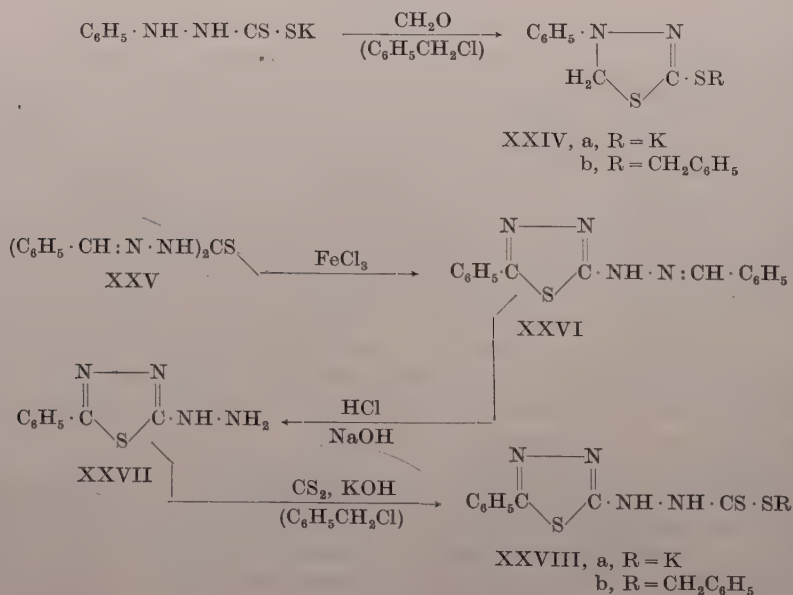
All three of the compounds (XXI) are remarkably stable to acids and bases. Long boiling in concentrated ammonia or sodium hydroxide solution was without effect, but on prolonged boiling with hydrazine hydrate one mole of the corresponding mercaptan was eliminated and a thiohydrazide was formed. The presence of the -CS·NH·NH₂ group was demonstrated by reaction with benzaldehyde and with carboxymethyl dithiobenzoate. In the first case a hydrazone was formed, and in the second the product was a 5-phenyl-1,3,4-thiadiazol-2-yl derivative. This thiadiazole derivative was also formed when the benzaldehyde hydrazone was oxidized with iron (III) chloride. These reactions prove the presence of a dithioester group in the compounds (XXI). Their formula can be written RS·C₂H₂N₂S·CS₂R, and the stability of the compounds, particularly towards nucleophilic reagents, suggests a cyclic structure with the dithioester group attached to a nitrogen atom. The compounds (XXI) are tentatively formulated as 2-R-thio- Δ^2 -1,3,4-thiadiazoline-4-dithiocarboxylates. The corresponding thiohydrazides then become (XXII) and the phenylthiadiazolyl derivative (XXIII).

The thiohydrazide (XXIIa) has m.p. 182°–183°, (XXIIb) 147°–148° and (XXIIc) 143°–144°. The benzaldehyde hydrazone of (XXIIa) has m.p. 183°–184°, of (XXIIb) 162°–163° and of (XXIIc) 164°–165°. The phenylthiadiazolyl derivative (XXIII) has only been prepared from (XXIIc) and has m.p. 129°–130°.

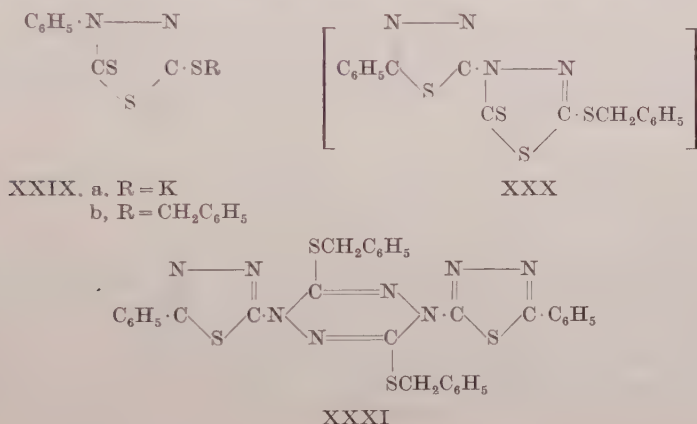


Attempts have been made to prepare the compound (XXIII) in an unambiguous way in order to prove the structure of (XXI). BUSCH (18) has shown that potassium 1-phenyldithiocarbazate reacts with formaldehyde to give the potassium salt of 2-mercapto-4-phenyl- Δ^2 -1,3,4-thiadiazoline (XXIV a), and this can react with benzyl chloride to (XXIV b).

Dibenzaldehyde thiocarbhydrazone (XXV) was oxidized with iron (III) chloride and gave a high yield of benzaldehyde 5-phenyl-1,3,4-thiadiazol-2-yl-hydrazone (XXVI), m.p. 256°–257°. This hydrazone was hydrolyzed with hydrochloric acid to the hydrochloride of the hydrazine (XXVII), and the free hydrazine, m.p. 190°–191°, was obtained when a solution of the hydrochloride was neutralized with sodium hydroxide. The preparation of the hydrazine (XXVII) by other methods has recently been described by FÜJII *et al.* (19). This hydrazine reacted with carbon disulphide and potassium hydroxide and gave potassium 1-[5'-phenyl-1',3',4'-thiadiazol-2'-yl]-dithiocarbazate (XXVIII a). This salt reacted rapidly with formaldehyde, but the resulting product was obviously polymeric, and no definable compound could be obtained from it. The benzyl ester (XXVIII b) was obtained from the potassium salt by reaction with benzyl chloride and had m.p. 194°–195°. It could not be made to react with formaldehyde to give (XXIII).



BUSCH has also shown (20) that phenylhydrazine when refluxed with an excess of potassium hydroxide and carbon disulphide gives the potassium salt of 2-mercapto-4-phenyl-1,3,4-thiadiazole-5(4H)-thione, (XXIXa), and this salt can react with benzyl chloride to give (XXIXb). It was thought that a similar series of reactions could be performed with the hydrazine (XXVII) to give the thiadiazole-thione (XXX), which might be hydrogenated to (XXIII) or to a hydrogenated derivative thereof, which might then also be obtained by hydrogenation of (XXIII). However, when the hydrazine (XXVII) was refluxed with an excess of carbon disulphide and potassium hydroxide a potassium salt was obtained, which with benzyl chloride gave a compound, m.p. 125°–126°, tentatively formulated as 1,4-di-[5'-phenyl-1',3',4'-thiadiazol-2'-yl]-3,6-dibenzylthio-1,4-dihydro-1,2,4,5-tetrazine (XXXI).



II. Experimental

A. Formaldehyde dithiocarbhydrazones

When 1.5 g of aqueous formaldehyde solution were added to a solution of 2.5 g of methyldithiocarbhydrazide (21) in 20 ml of *N*-NaOH, a clear solution was obtained. On the following day the mixture was acidified with 4 ml of 5 *N*-HCl, and the resulting oil was taken up in 100 ml of ether. The ether solution was dried with sodium sulphate, and 6 ml of 5 *N*-HCl in absolute ethanol were added. The mixture immediately began to deposit colourless flakes, 2.7 g with m.p. 116°–117°, according to analyses consisting of formaldehyde methyldithiocarbhydrazone hydrochloride.

For $\text{C}_3\text{H}_7\text{N}_2\text{S}_2\text{Cl}$ (170.69)

calc.: C 21.1 H 4.13 N 16.41 S 37.6 Cl 20.8 %,
found: C 20.8*¹ H 4.11* N 16.54* S 37.4² Cl 21.3* %.

From this hydrochloride 0.51 g was stirred with 5 ml of ethanol and 3 ml of 2 *M*-FeCl₃ solution. A clear, brown mixture was obtained. On the following day the solvent

¹ The analyses marked with an asterisk have been performed at the Analytical Department of the University of Lund, the rest, with a few exceptions, by the author.

² By A. BERNHARDT, Mikroanalytisches Laboratorium, Mülheim (Ruhr).

was distilled off and the residue was subjected to vacuum-sublimation at 150° and 2 mm Hg. The result was 0.25 g of colourless, rod-shaped prisms, m.p. 162°–163°. According to analyses the product was 2-methylthio-1,3,4-thiadiazole (IXa) hydrochloride.

For $C_5H_5N_2S_2Cl$ (168.67)

calc.: C 21.4 H 2.99 N 16.61 S 38.0 Cl 21.0 %,
found: C 21.3* H 3.12* N 16.76* S 37.8¹ Cl 20.8* %.

When an aqueous solution of this hydrochloride was neutralized with *N*-NaOH the free thiadiazole was obtained as a colourless oil, which could not be made to crystallize.

The formaldehyde benzyldithiocarbhydrazone hydrochloride was prepared in the same way as the corresponding methyl derivative. From 2.0 g of benzyldithiocarbhydrazone (21) and 0.75 g of 40 % aqueous formaldehyde solution 1.9 g of the hydrochloride were obtained as colourless, oblique plates, m.p. 115°–117°.

For $C_9H_{11}N_2S_2Cl$ (246.77)

calc.: C 43.8 H 4.49 N 11.35 S 26.0 Cl 14.37 %,
found: C 43.4* H 4.52* N 11.43* S 25.9¹ Cl 14.49* %.

From this hydrochloride 0.5 g was stirred with 10 ml of ethanol and 2 ml of 2 *M*-FeCl₃. A cloudy mixture was obtained, and on the following day 30 ml of water were added. A brown oil separated, which slowly solidified to 0.37 g of a sticky crystalline mass. The product was dissolved in benzene and subjected to chromatography on alumina. Elution with benzene gave first a brown oil, 0.06 g, and then 0.28 g of colourless flakes, m.p. 59°–60°. According to analyses the product was 2-benzylthio-1,3,4-thiadiazole (IXb).

For $C_9H_8N_2S_2$ (208.29)

calc.: C 51.9 H 3.87 N 13.45 S 30.8 %,
found: C 52.0* H 3.88* N 13.42* S 30.8* %.

This compound has recently been described by YOUNG and WOOD (8), who report m.p. 60.5°–62°.

B. Compound A and comparison compounds

a) Compound A

When 3.0 g of 40 % aqueous formaldehyde solution were added to a solution of 2.5 g of methylthiocarbhydrazone in 10 ml of ethanol, the mixture immediately became cloudy. On the following day a colourless oil had separated, which increased in amount on the addition of 40 ml of water. The oil was taken up in 50 ml of benzene, and the benzene solution was extracted with 20 ml of *N*-NaOH. On acidification with *N* acetic acid the alkaline solution deposited a small amount of colourless oil; the benzene solution on free evaporation yielded 2.2 g of a colourless oil, which slowly solidified to a crystalline mass, m.p. 43°–45°. The product was dissolved in 5 ml of

¹ By A. BERNHARDT.

ethyl acetate and 10 ml of ethanol and cooled to -5° . Careful addition of 10 ml of water precipitated 1.8 g of colourless, irregular prisms, m.p. 47° – 48° .

For $C_7H_{12}N_4S_4$ (280.44) (Compound A)

calc.: C 30.0 H 4.31 N 20.0 S 45.7 %, M: 280,

found: C 29.8 H 4.26 N 20.0 S 45.7 %, M: 291 (in benzene).

Formaldehyde methylthiocarbhydrazide hydrochloride, 0.34 g, was stirred with 5 ml of *N*-NaOH. The mixture was never clear, but a colourless oil remained undissolved. The latter slowly solidified to 0.15 g of a crystalline mass with m.p. 45° – 46° , which was undepressed on admixture with compound A.

When 1.0 g of 100 % hydrazine hydrate was added to a solution of 2.8 g of compound A in 15 ml of absolute ethanol and 15 ml of benzene, a clear, colourless mixture was obtained. On the following day a small amount of crystals had separated, and free evaporation yielded a residue of 2.0 g of a slightly sticky crystalline mass. Recrystallization from 10 ml of water yielded 1.4 g of colourless, rod-shaped prisms, m.p. 168° – 169° (decomp.), containing 30.3 % S. Thiocarbhydrazide requires 30.2 % S.

b) 2-Methyl-methylthiocarbhydrazide and its hydrazones

Methylhydrazine sulphate, 1.44 g, was dissolved in 10 ml of 2 *N*-NaOH and added to a solution of 1.38 g of dimethyl trithiocarbonate in 30 ml of ethanol. The mixture soon became colourless and smelt strongly of methyl mercaptan. On the following day the ethanol was distilled off under reduced pressure, and a water-insoluble residue of 1.10 g of colourless prisms was obtained, m.p. 92° – 94° . The product was recrystallized from 10 ml of carbon tetrachloride and yielded 0.85 g of colourless, thick, rectangular plates, m.p. 93° – 94° , according to analyses consisting of 2-methyl-methylthiocarbhydrazide (XI).

For $C_3H_8N_2S_2$ (136.23)

calc.: C 26.5 H 5.92 N 20.6 S 47.1 %,

found: C 26.6* H 5.93* N 20.5* S 47.0* %.

From this hydrazide 0.7 g was ground in a mortar with 5 ml of 2 *N*-HCl, and 1 g of solid sodium nitrite was added in portions. A yellow oil resulted which was separated, dissolved in 5 ml of ethanol and added to 0.25 ml of 100 % hydrazine hydrate. A colourless mixture was obtained, which was heated on a water bath for ten minutes. It smelt strongly of methyl mercaptan, and when the solvent was evaporated a residue of 0.33 g of a pink, sticky crystalline mass was obtained. This was dissolved in the smallest amount of ethanol and subjected to chromatography on alumina. Elution with benzene gave 0.11 g of a red oil, which was discarded, and then washing with ethanol gave 0.15 g of a colourless, crystalline mass, m.p. 133° – 135° alone and 135° – 136° on admixture with pure 4-methylthiosemicarbazide. The product was dissolved in 5 ml of 50 % aqueous ethanol, and 0.15 g of benzaldehyde was added. The mixture immediately began to deposit pale yellow crystals, on the following day 0.23 g, m.p. 159° – 160° . According to analyses the product was benzaldehyde 4-methylthiosemicarbazone.

For $C_9H_{11}N_3S$ (193.26)

calc.: C 55.9 H 5.74 N 21.7 S 16.59 %,

found: C 56.2* H 5.66* N 21.5* S 16.49* %.

For 4-methylthiosemicarbazide PULVERMACHER (11) reports m.p. 137° – 138° and for benzaldehyde 4-methylthiosemicarbazone 160° .

A solution of 0.54 g of the hydrazide (XI) in 4 ml of ethanol was added to 0.4 ml of 40 % aqueous formaldehyde in 4 ml of water. On the following day 0.48 g of colourless, rod-shaped prisms had separated, m.p. 64° – 65° . The product was recrystallized from 6 ml of 50 % aqueous ethanol and gave 0.36 g of colourless, rod-shaped prisms, m.p. 65° – 66° . According to analyses the product was formaldehyde 2-methyl-methyldithiocarbhydrazone (X).

For $C_4H_8N_2S_2$ (148.24)

calc.: C 32.4 H 5.44 N 18.90 S 43.3 %, M: 148,

found: C 32.5* H 5.51* N 18.90* S 43.2* %, M: 150 (in benzene).

A solution of 0.54 g of the hydrazide (XI) in 4 ml of ethanol was added to 2 ml of 10 % aqueous acetaldehyde solution. The mixture immediately thickened to a porridge of 0.60 g of colourless, long plates, m.p. 57° – 58° . Recrystallization from 6 ml of 70 % aqueous ethanol yielded 0.48 g of the acetaldehyde 2-methyl-methyldithiocarbhydrazone (XV) as long, colourless plates of unchanged m.p.

For $C_5H_{10}N_2S_2$ (162.27)

calc.: C 37.0 H 6.21 N 17.26 S 39.5 %,

found: C 37.0* H 6.20* N 17.30* S 39.6* %.

Benzaldehyde, 0.44 g, was added to a solution of 0.54 g of the hydrazide (XI) in 5 ml of ethanol. The mixture was clear until the following day, when the addition of 5 ml of water precipitated 0.60 g of pale yellow, rod-shaped prisms, m.p. 90° – 92° . Recrystallization from 6 ml of ethanol yielded 0.42 g of pale yellow, rod-shaped prisms, m.p. 92° – 93° , according to analyses consisting of benzaldehyde 2-methyl-methyldithiocarbhydrazone.

For $C_{10}H_{12}N_2S_2$ (224.34)

calc.: C 53.5 H 5.39 N 12.49 S 28.6 %,

found: C 53.7¹ H 5.40¹ N 12.53¹ S 28.6¹ %.

c) *Dibenzaldehyde S'-methylene-bis-S''-methyl-isodithiocarbhydrazone*

Methylene dibromide, 1.0 g, was added to a solution of 2.1 g of benzaldehyde methyldithiocarbhydrazone (21) in 10 ml of ethanol and 10 ml of *N*-NaOH. After about ten minutes an emulsion began to form, and on the following day 1.5 g of pale yellow, long plates had separated, m.p. 115° – 120° . The product was recrystallized twice from a mixture of 5 ml of benzene and 20 ml of absolute ethanol and yielded 0.7 g of pale yellow, rod-shaped prisms, m.p. 130° – 131° .

For $C_{18}H_{20}N_4S_4$ (432.62) (XIII)

calc.: C 52.8 H 4.66 N 12.95 S 29.6 %, M: 433,

found: C 52.9* H 4.64* N 12.97 S 29.8* %, M: 442 (in benzene).

¹ By Drs. WEYLER and STRAUSS, Oxford.

d) *Acetaldehyde methylthiocarbhydrazone and its disulphide*

When 25 ml of a 10% aqueous acetaldehyde solution were added to a solution of 6.2 g of methylthiocarbhydrazone in 50 ml of ethanol, a clear, colourless mixture was obtained. After about an hour colourless, needle-shaped prisms began to separate, and on the following day the addition of 100 ml of water completed the separation. The yield of acetaldehyde methylthiocarbhydrazone was 5.5 g of needles, m.p. 90°–91°. Recrystallization from 75 ml of ligroin (b.p. 80°–100°) yielded 4.6 g of colourless needles, m.p. 92°–93°.

For $C_4H_8N_2S_2$ (148.24)

calc.: C 32.4 H 5.44 N 18.90 S 43.3 %,

found: C 32.4 H 5.42 N 18.91 S 43.1 %.

A solution of 1.3 g of iodine in 15 ml of ethanol was added to a solution of 1.5 g of acetaldehyde methylthiocarbhydrazone in 10 ml of *N*-NaOH and 10 ml of ethanol. The mixture rapidly became cloudy, and on the following day 1.0 g of pale yellow flakes had separated, m.p. 105°–107°. Recrystallization from 10 ml of ligroin (b.p. 80°–100°) yielded 0.7 g of pale yellow, coarse prisms, m.p. 110°–111°. According to analyses the product was acetaldehyde methyl-*isodithiocarbhydrazone* disulphide (XIV).

For $C_8H_{14}N_4S_4$ (294.46)

calc.: C 32.6 H 4.79 N 19.03 S 43.6 %, M: 294,

found: C 32.7 H 4.78 N 18.97 S 43.4 %, M: 303 (Rast).

e) *Hydrogenation of 2-methylthio- and 2-benzylthio-4-methyl-1,3,4-thiadiazole-5-(4H)-thione*

A solution of 3.8 g of 2-methylthio-4-methyl-1,3,4-thiadiazole-5(4H)-thione (XVIb, 14) in 100 ml of absolute ether was added in portions to a suspension of 1.0 g of lithium aluminium hydride in 50 ml of absolute ether. After four hours water was added, the ether solution was separated and the ether was distilled off. The remaining slightly sticky, crystalline mass was purified by vacuum sublimation at 100° and 10 mm Hg. The yield was 2.5 g of colourless, pointed prisms, m.p. 95°–96°, according to analyses consisting of 2-methylthio-4-methyl-1,3,4-thiadiazolidine (XVIIIa).

For $C_4H_{10}N_2S_2$ (150.26)

calc.: C 32.0 H 6.71 N 18.64 S 42.7 %, M: 150,

found: C 32.3* H 6.70* N 18.70* S 42.8* %, M: 157 (in benzene).

The benzylthio compound (XVIc) has not been described earlier. It was obtained when 1.3 g of benzyl chloride in 10 ml of ethanol were added to a solution of 1.65 g of 2-mercapto-4-methyl-1,3,4-thiadiazole-5(4H)-thione (XVIa, 14) in 10 ml of *N*-NaOH. On the following day the mixture had deposited 2.4 g of a colourless crystalline mass, m.p. 73°–74°. Recrystallization from 20 ml of ethanol yielded 2.2 g of long, colourless plates, m.p. 75°–76°.

For $C_{10}H_{10}N_2S_2$ (254.38) (XVIc)

calc.: C 47.2 H 3.96 N 11.01 S 37.8 %,

found: C 47.3* H 4.04* N 11.03* S 37.9* %.

A solution of 2.5 g of this compound in 150 ml of absolute ether was added in portions to a suspension of 0.6 g of lithium aluminium hydride in 50 ml of absolute ether. After four hours water was added, the ether solution was separated and the ether was distilled off. A residue of 2.0 g of a colourless crystalline mass was obtained, m.p. 74°–76°. Recrystallization from 20 ml of ligroin (80°–100°) yielded 1.6 g of colourless, oblique prisms, m.p. 78°–79°. According to analyses the compound was 2-benzylthio-4-methyl-1,3,4-thiadiazolidine (XVIIIb).

For $C_{10}H_{14}N_2S_2$ (226.35)

calc.: C 53.1 H 6.23 N 12.38 S 28.3 %, M: 226,

found: C 53.2* H 6.19* N 12.44* S 28.2* %, M: 220 (in exaltone).

f) *Hydrogenation of compound A*

Compound A, 2.8 g, was dissolved in 50 ml of absolute ether and the solution was added in portions to a suspension of 1.0 g of lithium aluminium hydride in 50 ml of absolute ether. After four hours water was added, the ether solution was separated and the ether was distilled off. A residue of 1.3 g of a colourless oil was obtained, which was dissolved in 20 ml of absolute ether. The addition of 10 ml of 2 N-HCl in absolute ethanol caused a colourless oil to separate, and on standing overnight in a refrigerator this solidified to 0.50 g of a colourless crystalline mass, m.p. 130°–136°. It contained 19.1 % Cl, whereas the hydrochloride of (XVIIIa) requires 18.9 %. When the salt, 0.45 g, was stirred with 5 ml of water, a colourless oil separated, which soon solidified to 0.31 g of a colourless crystalline mass, m.p. 90°–93°. Vacuum sublimation yielded 0.27 g of colourless, pointed prisms with m.p. 95°–96°, which was undepressed on admixture with 2-methylthio-4-methyl-1,3,4-thiadiazolidine (XVIIIa) (p. 267).

For $C_4H_{10}N_2S_2$ (150.26)

calc.: C 32.0 H 6.71 N 18.64 S 42.7 %,

found: C 32.0* H 6.67* N 18.54* S 42.6* %.

g) *Reaction of compound A and its benzyl analog with methyl iodide*

Methyl iodide, 3.0 g, was added to a solution of 2.8 g of compound A in 30 ml of ethyl acetate. On the following day the mixture had deposited 3.4 g of pale brown flakes, m.p. 165°–166° (decomp.). Recrystallization from 100 ml of absolute ethanol yielded 2.6 g of colourless plates, m.p. 173°–174° (decomp.).

For $C_5H_{11}N_2S_2J$ (290.20) (XXa)

calc.: C 20.7 H 3.82 N 9.65 S 22.1 J 43.7 %,

found: C 20.6 H 3.78 N 9.61 S 22.2 J 43.6 %.

Determinations of the freezing point of solutions of the methiodide in water gave an apparent molecular weight of 145, which is the value required by the above formula.

The methiodide, 1.5 g, was heated with 10 ml of water to boiling under reflux, and 5 ml of 50 % aqueous sodium hydroxide were added. The gases passing through the condensor were bubbled through a U-tube with 5 ml of 8 N-HCl in absolute ethanol. This solution was evaporated to dryness, and a residue of 0.30 g of a colourless, hygroscopic crystalline mass was obtained. This was shaken with 1.0 g of *p*-toluenesulphonyl chloride in 15 ml of *N*-NaOH and finally boiled under reflux for ten minutes. After cooling, the resulting oil solidified to 0.69 g of a colourless

crystalline mass, m.p. 80° – 82° . Recrystallization from 10 ml of ligroin (80° – 100°) yielded 0.52 g of colourless, rod-shaped prisms, m.p. 81° – 82° . Authentic N,N-dimethyl-*p*-toluenesulphonamide was prepared from dimethylamine and *p*-toluenesulphonyl chloride and showed m.p. 81° – 82° after several recrystallizations. A mixed m.p. of these two samples showed no depression.

For $C_9H_{13}O_2NS$ (199.26) calc.: N 7.03 %, found: N 7.03* %.

For this sulphonamide STEINKOPF (22) reports m.p. 86° – 87° and CLARKE *et al.* (23) 78° – 79° .

The alkaline solution from the decomposition of the methiodide on acidification evolved hydrogen sulphide and carbon dioxide. When the alkaline decomposition was performed in a test-tube, a strong smell of methyl mercaptan was noticed.

A mixture of 7.5 g of 40 % aqueous formaldehyde solution and 30 ml of ethanol was added to a solution of 10 g of benzyldithiocarbhydrazide in 250 ml of ethanol. On the following day a clear, colourless oil had separated. The solution was evaporated to about 15 g, the oil was dissolved in 150 ml of ether and the ether solution was extracted with 50 ml of *N*-NaOH. The alkaline extract on acidification deposited a small amount of yellow oil. The ether solution was dried with sodium sulphate and 20 g of methyl iodide were added. On the following day the mixture had deposited 8.9 g of pale brown flakes, m.p. 156° – 157° (decomp.). Recrystallization from 150 ml of absolute ethanol yielded 7.6 g of colourless plates, m.p. 158° – 159° (decomp.).

For $C_{11}H_{15}N_2S_2J$ (366.29) (XXb)

calc.: C 36.1 H 4.13 N 7.65 S 17.51 J 34.7 %,

found: C 36.3 H 4.10 N 7.61 S 17.45 J 34.5 %.

When this methiodide was subjected to alkaline decomposition under the same conditions as the methyl derivative (XXa) nearly the same yield of N,N-dimethyl-*p*-toluenesulphonamide was obtained. The alkaline mixture from the decomposition contained a colourless oil, which on cooling solidified to a crystalline mass, m.p. 66° – 68° . The yield from 1.8 g of the methiodide was 0.57 g. Recrystallization from 4 ml of ethanol gave 0.42 g of colourless plates with m.p. 70° – 71° , which was undepressed on admixture with pure dibenzyl disulphide.

For $C_{14}H_{14}S_2$ (246.37)

calc.: C 68.2 H 5.73 S 26.0 %,

found: C 68.0 H 5.74 S 26.2 %.

C. Thiadiazoline-4-dithiocarboxylates and their derivatives

A mixture of 3.2 g of 40 % aqueous formaldehyde and 10 ml of ethanol was added to a solution of 10.0 g of *methyldithiocarbhydrazide* in 20 ml of ethanol and 40 ml of 5 *N*-HCl. After two weeks the mixture had deposited 4.9 g of colourless, irregular prisms, m.p. 140° – 141° . Recrystallization from 70 ml of ethyl acetate yielded 4.2 g of colourless, irregular prisms, m.p. 143° – 144° . According to analyses the product was methyl 2-methylthio- Δ^2 -1,3,4-thiadiazoline-4-dithiocarboxylate (XXIa).

For $C_6H_8N_2S_4$ (224.37)

calc.: C 26.8 H 3.59 N 12.49 S 57.2 %, M: 224,

found: C 26.8 H 3.60 N 12.61 S 57.0 %, M: 230 (Rast).

The mother liquor from the crude product was evaporated to a yellow oil and a crust of colourless crystals. The crystals were washed free from oil with benzene and then dissolved in 20 ml of water. Addition of 10 g of benzaldehyde in 40 ml of ethanol to the water solution caused the separation of 8.3 g of yellow, irregular prisms, melting between 70° and 130°. The crystals were ground with 40 ml of *N*-NaOH, and a residue of 6.3 g of a yellow crystalline powder was obtained, m.p. 88°–90°. Recrystallization from 15 ml of ethanol yielded 5.4 g of yellow, rod-shaped prisms with m.p. 92°–93°, which was undepressed on admixture with pure benzalazine. The alkaline extract on acidification deposited 2.0 g of a pale yellow crystalline powder with m.p. 157°–158°, which was undepressed on admixture with pure benzaldehyde methylthiocarbhydrazone.

Formaldehyde methylthiocarbhydrazone hydrochloride, 0.37 g, was dissolved in 5 ml of boiling absolute ethanol. On cooling and after three days the mixture had deposited 0.15 g of colourless, irregular prisms, m.p. 142°–143°, undepressed on admixture with (XXIa).

Compound A, 1.4 g, was dissolved in 50 ml of ethanol, and 10 ml of 5 *N*-HCl in absolute ethanol was added. After three days the solution was evaporated to a sticky mass. This was stirred with 5 ml of ethanol and gave a residue of 0.35 g of colourless crystal powder, m.p. 140°–142°. Recrystallization from 5 ml of ethyl acetate gave 0.28 g of colourless, irregular prisms, m.p. 143°–144°, undepressed on admixture with (XXIa).

For $C_5H_8N_2S_4$ (224.37) calc.: S 57.2 %, found: S 56.9* %.

A solution of 2.2 g of the dithioester (XXIa) in 100 ml of benzene and 100 ml of absolute ethanol was refluxed for three hours with 1.0 ml of 100 % hydrazine hydrate. Then the solvent was distilled off until about 25 ml remained. On cooling, the mixture deposited 1.7 g of reddish prisms, m.p. 175°–176° (decomp.). The product could not be recrystallized, but after extraction with 15 ml of boiling benzene in portions 1.3 g of colourless prisms remained undissolved, m.p. 182°–183° (decomp.), according to analyses consisting of 2-methylthio- Δ^2 -1,3,4-thiadiazoline-4-thiocarboxhydrazone (XXIIa).

For $C_4H_8N_4S_3$ (208.31)

calc.: C 23.1 H 3.87 N 26.9 S 46.2 %,

found: C 23.3 H 3.77 N 27.0 S 45.8 %.

From this hydrazide 0.21 g was refluxed in 10 ml of glacial acetic acid with 0.11 g of benzaldehyde for five minutes, when the mixture was clear. On cooling no deposition occurred, but when the mixture was poured into 200 ml of water 0.25 g of pale yellow hairs was precipitated, m.p. 180°–182°. Recrystallization from 20 ml of toluene gave 0.20 g of pale yellow hairs, m.p. 183°–184°, according to analyses consisting of the benzaldehyde hydrazone of (XXIIa).

For $C_{11}H_{12}N_4S_3$ (296.42)

calc.: C 44.6 H 4.08 N 18.90 S 32.4 %,

found: C 45.0* H 4.15* N 18.75* S 32.4* %.

A mixture of 1.5 g of 40 % aqueous formaldehyde and 10 ml of ethanol was added to a solution of 2.7 g of *ethylthiocarbhydrazone* in 10 ml of ethanol and 5 ml of 5 *N*-HCl. A clear mixture was obtained, which after a week had deposited 1.5 g of long, colourless plates, m.p. 78°–79°. Recrystallization from 7 ml of absolute ethanol gave

1.2 g of long, colourless plates of unchanged m.p., according to analyses consisting of ethyl 2-ethylthio- Δ^2 -1,3,4-thiadiazoline-4-dithiocarboxylate (XXIb).

For $C_7H_{12}N_2S_4$ (252.43)

calc.: C 33.3 H 4.79 N 11.10 S 50.8 %, M: 252,
found: C 33.4* H 4.86* N 11.16 S 51.1* %, M: 240 (Rast).

From this dithioester 2.5 g were refluxed in 25 ml of ethanol with 1.0 ml of 100 % hydrazine hydrate for three hours. On cooling, the mixture deposited 1.6 g of reddish, rod-shaped prisms, m.p. 143° – 145° . Recrystallization from 20 ml of toluene gave 1.3 g of colourless, rod-shaped prisms, m.p. 147° – 148° , according to analyses consisting of 2-ethylthio- Δ^2 -1,3,4-thiadiazoline-4-thiocarboxhydrazide (XXIIb).

For $C_8H_{10}N_4S_3$ (222.34)

calc.: C 27.0 H 4.53 N 25.2 S 43.3 %,
found: C 27.1* H 4.60* N 25.1 S 43.4* %.

Benzaldehyde, 0.11 g, was added to a solution of 0.22 g of this thiohydrazide in 4 ml of hot glacial acetic acid. On the following day 0.20 g of pale yellow, thick plates had separated, m.p. 160° – 162° . Recrystallization from 2 ml of toluene yielded 0.12 g of pale yellow, thick plates, m.p. 162° – 163° . According to analyses the product was the benzaldehyde hydrazone of (XXIIb).

For $C_{12}H_{14}N_4S_3$ (310.44)

calc.: C 46.4 H 4.55 N 18.05 S 31.0 %,
found: C 46.7* H 4.65* N 17.95* S 30.8* %.

Benzylidithiocarbhydrazide, 16.0 g, was dissolved in 50 ml of 5 N-HCl and 100 ml of ethanol, and a 10 % aqueous formaldehyde solution was added by drops with stirring, until the cloudiness induced by every drop did not disappear on stirring. Then 2.0 g of formaldehyde had been added, which corresponds to a molar ratio for formaldehyde : benzylidithiocarbhydrazide = 0.83. If more formaldehyde was added, the yield and purity of the product decreased. The mixture slowly began to deposit pale yellow, needle-shaped prisms. After two weeks the yield was 10.5 g with m.p. 103° – 105° . The product was recrystallized twice from a mixture of 10 ml of benzene and 40 ml of absolute ethanol and gave 8.7 g of pale yellow, needle-shaped prisms, m.p. 110° – 111° , according to analyses consisting of benzyl 2-benzylthio- Δ^2 -1,3,4-thiadiazoline-4-dithiocarboxylate (XXIc).

For $C_{17}H_{16}N_2S_4$ (376.56)

calc.: C 54.2 H 4.28 N 7.44 S 34.1 %, M: 377,
found: C 54.5 H 4.32 N 7.47 S 34.2 %, M: 368 (Rast).

When the mother liquor from the crude product was treated as in the experiment with methylidithiocarbhydrazide (p. 270), a yield of 4.5 g of pure benzalazine was finally obtained.

Formaldehyde benzylidithiocarbhydrazone hydrochloride, 0.5 g, was dissolved in 10 ml of boiling absolute ethanol. On cooling and after three days the mixture had deposited 0.22 g of pale yellow, needle-shaped prisms, m.p. 110° – 111° , undepressed on admixture with (XXIc).

A solution of 7.5 g of the dithioester (XXIc) in 100 ml of benzene and 100 ml of absolute ethanol was refluxed for three hours with 2.0 ml of 100 % hydrazine hydrate.

Then the solvents were distilled off until about 40 ml remained. On cooling, the mixture deposited 4.6 g of colourless plates, m.p. 142°–143°. Recrystallization from 250 ml of ethanol yielded 3.8 g of colourless plates, m.p. 143°–144°. According to analyses the product was 2-benzylthio- Δ^2 -1,3,4-thiadiazoline-4-thiocarboxhydrazide (XXIIc).

For $C_{10}H_{12}N_4S_3$ (284.41)

calc.: C 42.2 H 4.25 N 19.70 S 33.8 %,

found: C 42.3 H 4.27 N 19.77 S 33.7 %.

Benzaldehyde, 0.11 g, was added to a solution of 0.28 g of this thiohydrazide in 8 ml of hot glacial acetic acid. On cooling, the mixture deposited 0.34 g of pale yellow, rod-shaped prisms, m.p. 164°–165°. Recrystallization from a mixture of 20 ml of benzene and 20 ml of absolute ethanol gave 0.28 g of pale yellow, rod-shaped prisms of unchanged m.p. According to analyses the product was the benzaldehyde hydrazone of the thiohydrazide (XXIIc).

For $C_{17}H_{16}N_4S_3$ (372.51)

calc.: C 54.8 H 4.33 N 15.04 S 25.8 %,

found: C 55.0 H 4.34 N 15.02 S 25.9 %.

A solution of 2.1 g of carboxymethyl dithiobenzoate (24) in 10 ml of *N*-NaOH was added to a suspension of 2.8 g of the thiohydrazide (XXIIc) in 100 ml of ethanol. The mixture was refluxed for ten minutes, when a clear, red solution resulted. Hydrogen sulphide was evolved, and the red colour soon faded. On the following day the mixture had deposited 3.1 g of long, pale yellow plates, m.p. 123°–124°. Recrystallization from a mixture of 10 ml of benzene and 50 ml of absolute ethanol yielded 2.4 g of pale yellow, long plates, m.p. 129°–130°, according to analyses consisting of 2-benzylthio-4-[5'-phenyl-1',3',4'-thiadiazol-2'-yl]- Δ^2 -1,3,4-thiadiazoline (XXIII).

For $C_{17}H_{14}N_4S_3$ (370.49)

calc.: C 55.1 H 3.81 N 15.12 S 26.0 %, M: 370,

found: C 55.2 H 3.79 N 15.17 S 26.0 %, M: 380 (Rast).

The benzaldehyde hydrazone of (XXIIc), 0.37 g, was refluxed with 10 ml of ethanol and 1.0 ml of 2 *M*-FeCl₃ for ten minutes, when a clear solution resulted. On cooling, the solution deposited 0.31 g of pale yellow, long plates with m.p. 129°–130°, which was undepressed by (XXIII).

For $C_{17}H_{14}N_4S_3$ (370.49) calc.: C 55.1 H 3.81 %,

found: C 55.2* H 3.86* %.

D. Attempts to prepare 2-benzylthio-4-[5'-phenyl-1', 3', 4'-thiadiazol-2'-yl]- Δ^2 -1,3,4-thiadiazoline (XXIII)

a) Preparation of 5-phenyl-1,3,4-thiadiazol-2-yl-hydrazine

A suspension of 11.2 g of dibenzaldehyde thiocarbhydrazone (XXV, 25) in 200 ml of ethanol was refluxed for ten minutes with 40 ml of 2 *M*-FeCl₃. Then a clear solution resulted, which on cooling deposited 10.0 g of pale brown plates, m.p. 250°–252°. Recrystallization from 200 ml of glacial acetic acid yielded 8.8 g of pale yellow

plates, m.p. 256°–257°. According to analyses the product was benzaldehyde 5-phenyl-1,3,4-thiadiazol-2-yl-hydrazone (XXVI).

For $C_{15}H_{12}N_4S$ (280.34)

calc.: C 64.3 H 4.31 N 19.99 S 11.44 %.

found: C 64.4* H 4.35* N 19.97 S 11.45* %.

This hydrazone, 5.6 g. was suspended in 200 ml of concentrated hydrochloric acid and steam-distilled for ten hours. Benzaldehyde appeared in the distillate, and the remaining acid solution was filtered hot from a small amount of undissolved material. On cooling, a pale yellow, crystalline powder separated, 3.0 g, and by evaporating the mother liquor to dryness a further 0.5 g were obtained. No attempt was made to purify this hydrochloride, but the combined crops were dissolved in 50 ml of hot water, and the solution was neutralized against phenolphthalein with 16 ml of *N*-NaOH. The mixture immediately began to deposit grey flakes, and after cooling with ice the yield was 2.5 g, m.p. 180°–183°. The crude product was easily oxidized to a dark blue oil and had to be protected from prolonged contact with air. Recrystallization from 50 ml of ethanol gave 2.1 g of colourless plates, m.p. 190°–191°. Further recrystallization from ethanol did not change the m.p. The purified product is not oxidized in the air. According to analyses the product was 5-phenyl-1,3,4-thiadiazol-2-yl-hydrazine (XXVII).

For $C_8H_8N_4S$ (192.23)

calc.: C 50.0 H 4.19 N 29.1 S 16.68 %.

found: C 50.1* H 4.33* N 29.2* S 16.69* %.

For this compound FUJII *et al.* (19) report m.p. 185°–186°.

b) 1-[5'-phenyl-1',3',4'-thiadiazol-2'-yl]-benzylidithiocarbhydrazide

Carbon disulphide, 0.25 g in 3 ml of absolute ethanol, was added to a solution of 0.60 g of the hydrazine (XXVII) and 0.18 g of KOH in 15 ml of absolute ethanol and 15 ml of ether. The mixture was cooled with ice, and after a few minutes crystals began to form. After two hours the yield was 0.82 g of pale brown, rod-shaped prisms. The product was dissolved in 20 ml of water, and 0.33 g of benzyl chloride in 20 ml of ethanol was added. The mixture soon began to deposit crystals, and on the following day the yield was 0.68 g of a pale brown crystalline powder, m.p. 189°–191°. Recrystallization from a mixture of 10 ml of toluene and 10 ml of absolute ethanol yielded 0.48 g of colourless, rod-shaped prisms, m.p. 194°–195°, according to analyses consisting of 1-[5'-phenyl-1',3',4'-thiadiazol-2'-yl]-benzylidithiocarbhydrazide (XXVIII b).

For $C_{16}H_{14}N_4S_3$ (358.48)

calc.: C 53.6 H 3.94 N 15.63 S 26.8 %, M: 358,

found: C 53.6* H 3.94* N 15.74* S 26.7* %, M: 370 (Rast).

This dithiocarbhydrazide was treated with equivalent amounts and with excess of formaldehyde in neutral and acid solutions, but no reaction occurred. When a mixture of the dithiocarbhydrazide and excess of formaldehyde was evaporated to dryness on a water bath a sticky residue was obtained, but chromatography on

alumina showed that neither the thiadiazoline (XXIII) nor the starting material (XXVIIIb) was present in noticeable amounts.

When the potassium 1-[5'-phenyl-1', 3', 4'-thiadiazol-2'-yl]-dithiocarbazate (XXVIIIa) reacted with formaldehyde in aqueous solution, an amorphous, alkali-insoluble precipitate was obtained, from which no definable product could be isolated.

c) 1,4-Di-[5'-phenyl-1', 3', 4'-thiadiazol-2'-yl]-3,6-dibenzylthio-1,4-dihydro-1,2,4,5-tetrazine

A solution of 0.77 g of the hydrazine (XXVII), 1.2 g of carbon disulphide and 0.42 g of potassium hydroxide in 40 ml of ethanol was refluxed for two hours. On cooling, the mixture began to deposit crystals, which increased considerably in amount on the addition of 100 ml of ether. The yield was 0.40 g of pale yellow, rod-shaped prisms. The product was dissolved in 5 ml of water, and 0.20 g of benzyl chloride in 5 ml of ethanol was added. The mixture soon began to deposit crystals, and on the following day 0.35 g of pale yellow, rodshaped prisms had separated, m.p. 122°-124°. Recrystallization from 3 ml of ethanol yielded 0.28 g of colourless, rod-shaped prisms, m.p. 125°-126°. According to analyses the product was the above-mentioned dihydrotetrazine (XXXI).

For $C_{32}H_{24}N_8S_4$ (648.81)

Calc.: C 59.2 H 3.73 N 17.27 S 19.77 %,

found: C 59.3* H 3.74* N 17.26* S 19.75* %.

Light absorption measurements

The infra-red absorption spectra have been recorded with a Beckman IR-2 spectrophotometer with sodium chloride prism, cell-thickness 0.1 mm. The measurements have been performed with the compounds in 10 % solutions in carbon tetrachloride.

The ultra-violet absorption spectra have been recorded with a Beckman DU spectrophotometer and silica cells. Compound A was used in a 5×10^{-5} M solution in ethanol, the two other compounds in 10^{-4} M solutions in ethanol.

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Uppsala 1956. Almqvist & Wiksells Boktryckeri AB



Studies on the hydrolysis of metal ions

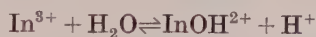
Part 14. The hydrolysis of the indium(III) ion, In^{3+}

By GEORGE BIEDERMANN

With 7 figures in the text

The reaction between the indium(III) ion and water was first considered in 1904 by Thiel [1] who explained the abnormal rise of the equivalent conductance of dilute indium chloride solutions by assuming that In^{3+} sets free hydrogen ions from water.

The first attempt to determine the composition of the hydrolysis products and the equilibrium constant of the reaction was made by Hattox and De Vries [2] in 1935. The pH of $\text{In}_2(\text{SO}_4)_3$ solutions was measured with a quinhydrone electrode. The equilibrium



was assumed, but the calculated constant varied considerably in the concentration range studied. In 1941–42 Moeller [3, 4] measured the pH of purified indium halide solutions with a glass electrode. The data were explained assuming the same equilibrium as Hattox and De Vries with the constants $1.2 \times 10^{-5} \text{ M}$ in chloride, $1.4 \times 10^{-5} \text{ M}$ in bromide, $1.8 \times 10^{-5} \text{ M}$ in iodide solutions. A recalculation of these data by Hepler and Hugus [4a] has given a constant of $1.4 \times 10^{-4} \text{ M}$ at infinite dilution. A similar recalculation of the data of Hattox and De Vries [2] has given the value of $1.25 \times 10^{-4} \text{ M}$ (F. Rossotti, private communication).

As was previously pointed out in this series [5], pH measurements of pure salt solutions cannot be considered a satisfactory method of studying the hydrolysis process since acid or basic impurities are very difficult to avoid in the preparation of the salt. Moreover this method of investigation only permits the study of a narrow pH range.

The presence of sulfate or halide ions is undesirable when the hydrolysis is to be investigated; these ions are known to form complexes with In(III) and in addition sulfate ion functions as a base.

Recently efforts have been made in this laboratory to work out suitable experimental methods of investigating complicated ionic equilibria; methods have been developed by Sillén [6, 7] of finding the composition of the reaction products and the corresponding formation constants from the experimental data.

The present work was undertaken to determine the mechanism of hydrolysis of the In(III) ion and the equilibrium constants of the consecutive steps of the process.

The method of investigation

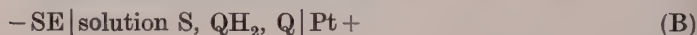
To study these equilibria, a number of potentiometric titrations were made. In each of these (corresponding to some twenty experimental points) the total concentration of In, B , was kept constant, whereas the hydrogen ion concentration, h , was varied. By emf methods h and $b = [\text{In}^{3+}]$ were measured. From these data the functions Z and η were calculated which are the basis for the following discussion.

In order to minimize the variation of the activity factors all solutions were made 3M with respect to perchlorate ion by the addition of NaClO_4 . The solutions thus had the general composition

$[\text{In(III)}]: B \text{ mM}, [\text{H}^+]: H \text{ mM}, [\text{Na}^+]: (3000 - 3B - H) \text{ mM}, [\text{ClO}_4^-]: 3 \text{ M} = \text{solution S}$

where mM denotes millimol/liter, B is the total concentration of In(III), and H is the "analytical" hydrogen ion concentration, assuming no hydrolysis.

To determine $[\text{In}^{3+}]$ and $[\text{H}^+]$ at equilibrium the cells



have been used. SE denotes the half-cell



The emf's of these cells at 25°C can be written

$$E_A = E_{\text{OA}} - 19.72 \log [\text{In}^{3+}] - 19.72 \log f_{\text{In}^{3+}} - E_j \quad (1)$$

$$E_B = E_{\text{OB}} + 59.16 \log h + 59.16 \log f_{\text{H}^+} + E_j + \delta \quad (2)$$

$$E_C = E_{\text{OC}} - 59.16 \log h - 59.16 \log f_{\text{H}^+} - E_j \quad (3)$$

where E_{OA} , E_{OB} and E_{OC} are constants, $f_{\text{In}^{3+}}$ and f_{H^+} denote the activity factors of In^{3+} and H^+ , E_j is the liquid junction potential between solutions S and 3M NaClO_4 , h is the equilibrium hydrogen ion concentration and δ is a small correction to account for the basic nature of quinone.

It was shown previously [8] that E_j is practically a function of h only. In cells (A) and (C) the equation $E_j = -0.0167h \text{ mV}$, and in cell (B) $E_j + \delta = -0.019h \text{ mV}$ was used.

The standard state is defined so that the activity factors tend to unity if the solutes are present in negligible concentrations in the solvent 3M NaClO_4 . Since the solutions are rather dilute in In^{3+} , H^+ and the reaction products, we may

assume the activity factors to be unity. It has been concluded elsewhere [8] that if B is low compared with $[\text{ClO}_4^-]$ the activity factors remain constant within the limits of experimental error until about 20 % of Na^+ is replaced by H^+ . Measurements now being carried out in this laboratory have shown that if h is kept constant and Na^+ is replaced by In^{3+} , $f_{\text{In}^{3+}}$ and f_{H^+} increase gradually with B . Within each series of measurements, in which B is kept constant and h is varied, the activity factors are certainly not influenced appreciably so that $[\text{In}^{3+}]$ and $[\text{H}^+]$ can be calculated from (1)–(3) neglecting the term f . The small variations of $f_{\text{In}^{3+}}$ and f_{H^+} will affect the value of the equilibrium constants calculated at different values of B . The equilibrium constants, however, could not be determined with so high an accuracy as to warrant the introduction of a correction term.

Each series of measurements (B constant, and h varied) consists of two parts. First the emf is determined at so high an acidity that the hydrolysis can be neglected; E_{OA} and E_{OB} or E_{OC} can then be calculated with (1)–(3). In the second part with further addition of NaHCO_3 the hydrolysis sets in, and since E_0 is known $[\text{In}^{3+}]$ and h can be obtained using (1)–(3).

Materials and analysis

Indium perchlorate solutions were prepared from indium metal of 99.95 % purity generously supplied and spectrographically examined by the Consolidated Mining and Smelting Company of Canada, Ltd.

Indium dissolves very slowly in hot concentrated HClO_4 and a considerable amount of Cl^- is formed. Presence of platinum accelerates the dissolution, but then the solution will be contaminated by Pt(IV) because a soluble Pt-In alloy is formed.

The metal was dissolved in concentrated HNO_3 , which had been purified by distillation. After dissolution a large excess of 70 % HClO_4 was added. HNO_3 and a large part of the excess HClO_4 was expelled by heating with an infra-red lamp. In the final product Cl^- , NO_3^- , SO_4^{2-} and Fe^{3+} could not be detected.

In(III) was determined as In_2S_3 , by the method of Thiel and Luckmann [9]. It was found convenient to precipitate In(III) with NH_3aq free from SiO_2 . The solubility of In(OH)_3 in alkaline solutions is inappreciable so the results are not influenced by the excess of NH_3 . The precipitate is filtered through a porcelain filter crucible containing an ash-free filter paper to hasten the filtration. After washing the precipitate with hot water it is dried at 120°C , and then ignited at $900\text{--}1000^\circ\text{C}$ for two hours. The crucible is cooled and placed in an oven at $350\text{--}400^\circ\text{C}$ and dry H_2S free from oxygen is led in for 30–45 minutes. A slow stream of CO_2 is passed to avoid oxidation during cooling. In_2S_3 has a constant weight under normal atmospheric conditions. This method of analysis was found to be accurate at least within $\pm 0.1\%$.

Attempts were made to determine In(III) as indium oxinate by the method of Geilmann and Wrigge [10]. No reproducible results could be obtained; at relatively high pHs the precipitate contained In(OH)_3 , and if the pH was lowered appreciable amounts of In(III) remained in solution.

The excess of hydrogen ion concentration in the indium perchlorate stock solution was determined using the ion exchange resin Duolite C3. The hydrogen

ion concentration of the eluate, which is equal to $[\text{ClO}_4^-]$, was determined by standard NaOH; the excess hydrogen ion concentration is given by $[\text{ClO}_4^-] - 3B$. This analysis was checked by the potentiometric titration described by Berecki-Biedermann [5].

Indium amalgam containing 2.00 % indium was made by dissolving freshly cut pieces of indium metal in distilled mercury. A two-limbed vessel was used to prepare the amalgam in the absence of oxygen. Weighed amounts of indium and mercury were placed in the separate limbs, the vessel was evacuated and then CO_2 free from O_2 was passed for about an hour. Finally the stopcocks were closed and the metal was dropped into the mercury by tilting the vessel. Oxide-free amalgams have a perfectly bright surface. A very sensitive test to detect oxidation is to observe the flow of the amalgam; if any trace of oxide is present it will adhere to the glass wall.

Perchloric acid solutions were standardized against KHCO_3 and Ti_2CO_3 , *sodium hydroxide* solutions against $\text{N}_2\text{H}_5\text{HSO}_4$ and potassium acid phthalate. The determinations agreed within 0.1 %.

Sodium hydrogen carbonate solutions were made from NaHCO_3 Merck p.a. containing less than 0.01 % Cl^- , and were standardized against HClO_4 .

Sodium perchlorate solutions were prepared from Na_2CO_3 (Cl^- -free) and HClO_4 . First a slight excess of HClO_4 was added and the solution was boiled to expel CO_2 . After cooling, the solution was carefully neutralized with NaOH. From the neutral solution a colloidal precipitate was formed, consisting of SiO_2 and traces of FeOOH . The precipitate could easily be removed by filtration after standing for 2-3 days. In the final product no traces of heavy metals or Cl^- could be detected, in a 6M NaClO_4 solution the SiO_2 content was less than 1mM, the $[\text{SO}_4^{2-}]$ was about 0.1mM. The solution was analyzed for NaClO_4 as described previously [11].

Sodium chloride Merck p.a. was used without further purification. It contained less than 0.01 % Br^- , since no Br^- could be detected using the eosin or BrCN methods.

Carbon dioxide and *nitrogen* were taken from cylinders, and freed from oxygen by passage over hot activated copper. Acid or basic impurities in nitrogen were absorbed in NaOH and H_2SO_4 solutions. The gases were finally passed through 3M NaClO_4 to obtain the correct vapor pressure.

Experimental details of the emf measurements

All emf measurements were made at $25.0 \pm 0.1^\circ\text{C}$.

The cell arrangement is described elsewhere [12]. The emf-s of cells (A) and (B) were measured with a Leeds and Northrup potentiometer of type K which was checked against a recently certified potentiometer of the Cambridge Instrument Company Ltd. The emf of cell (C) was measured with a valve potentiometer "Radiometer PHM3", Copenhagen, which was calibrated against the Leeds and Northrup potentiometer.

About 50-100 g indium amalgam was introduced into the cell from the two-limbed amalgam vessel under a strong stream of CO_2 ; at the same time N_2 passed through the solution. The electrical connection with the amalgam pool was made by a Pt needle.

In solutions of $B \geq 1\text{mM}$ the amalgam electrodes attained a steady potential

Table 1. Measurements with amalgam and glass electrodes.

 $B = 100.0 \text{ mM}, H = 50.00 \text{ mM}, [\text{ClO}_4^-] = 3 \text{ M}, 49.62 \text{ ml}$
 $B = 200.0 \text{ mM}, H = 61.86 \text{ mM}, [\text{ClO}_4^-] = 3 \text{ M}, v_1 \text{ ml}$
 $[\text{NaHCO}_3] = 115.3 \text{ mM}, [\text{ClO}_4^-] = 3 \text{ M}, v_2 \text{ ml}$
 $[\text{NaHCO}_3] = 246.8 \text{ mM}, [\text{ClO}_4^-] = 3 \text{ M}, v_3 \text{ ml}$
 $E_{\text{OA}} = 668.50 \pm 0.02 \text{ mV}$
 $E_{\text{OC}} = 133.8 \pm 0.1 \text{ mV}$

v_1 ml	v_2 ml	v_3 ml	E_A mV	E_C mV	H mM	h mM	b mM	E_{OA} mV	E_{OC} mV	E_j mV	Z	η
0.00	0.00		629.88	34.2	50.00			668.48	133.9	0.84		
5.00	5.00		629.70	41.6	37.08			668.52	133.8	0.62		
7.50	7.50		629.60	45.1	32.14			668.50	133.8	0.54		
9.00	9.00		629.55	47.3	29.53			668.50	133.8	0.49		
10.00	10.00		629.52	48.7	27.91			668.49	133.7	0.47		
12.00	12.00		629.47	51.2	24.93			668.49	(133.4)	0.42		
19.50	—	7.50	629.33	81.4	4.99	7.72	98.4			0.13	0.027	0.007
22.00		10.00	629.51	91.8	— 0.24	5.13	95.9			0.09	0.057	0.018
24.50		12.50	629.82	98.3	— 4.94	3.98	92.3			0.07	0.089	0.035
27.00		15.00	630.12	102.2	— 9.21	3.42	89.0			0.06	0.126	0.051
32.00		20.00	630.73	107.1	— 16.55	2.83	82.8			0.05	0.200	0.082
37.00		25.00	631.27	110.5	— 22.7	2.48	77.6			0.04	0.252	0.110
44.50		32.50	631.90	114.4	— 30.3	2.13	72.1			0.04	0.324	0.142
52.00		40.00	632.49	116.4	— 36.4	1.97	67.2			0.03	0.384	0.173
62.00		50.00	633.15	119.1	— 42.9	1.77	62.2			0.03	0.447	0.206
72.00		60.00	633.70	120.9	— 48.1	1.65	58.4			0.03	0.498	0.234
82.00		70.00	634.15	122.8	— 52.3	1.53	55.3			0.02	0.535	0.257

within 15 minutes and remained constant for at least 12 hours within 0.01–0.02 mV. For $B = 0.5 \text{ mM}$ about an hour was necessary to obtain equilibrium potentials. For $B \geq 1 \text{ mM}$ $E_A - E_{\text{OA}}$ values were reproducible usually within 0.05 mV, the maximum deviation was 0.1 mV. For $B = 0.5 \text{ mM}$ the reproducibility was $\pm 0.1 \text{ mV}$.

Quinhydrone electrodes functioned very satisfactorily in In(III) solutions, $E_B - E_{\text{OB}}$ was reproducible in general within $\pm 0.05 \text{ mV}$. The quinhydrone used was freed from acid impurities and traces of Fe^{3+} by recrystallization from water. The bright Pt foils were carefully cleaned with HNO_3 and then ignited in an alcohol flame before use.

Glass electrodes prepared for this laboratory by Mr. E. Blomgren, Uppsala, attained within 5–10 minutes a constant potential which did not change more than $\pm 0.2 \text{ mV}$ during several hours. $E_C - E_{\text{OC}}$ was reproducible within ± 0.2 – 0.4 mV .

When the same set of solutions was studied first with a glass electrode, and then with a quinhydrone electrode the difference kept constant within 0.1–0.2 mV. Thus no appreciable complex formation occurs between In(III) and quinhydrone or its dissociation products.

Ag, AgCl electrodes were prepared according to Brown [13]. The HCl solution used for electrolysis was freed from Br^- by adding several portions of KMnO_4 and expelling the Cl_2 and Br_2 after each addition by boiling. Finally the solution was distilled and the middle fraction was taken.

Table 2. Measurements with quinhydrone electrode.

$B = 1.017 \text{ mM}$, $H = 19.07 \text{ mM}$, $[\text{ClO}_4^-] = 3 \text{ M}$, 49.62 ml
 $B = 2.033 \text{ mM}$, $H = 2.86 \text{ mM}$, $[\text{ClO}_4^-] = 3 \text{ M}$, $v_1 \text{ ml}$
 $[\text{NaHCO}_3] = 47.04 \text{ mM}$, $[\text{ClO}_4^-] = 3 \text{ M}$, $v_2 \text{ ml}$
 $[\text{NaHCO}_3] = 11.78 \text{ mM}$, $[\text{ClO}_4^-] = 3 \text{ M}$, $v_3 \text{ ml}$
 $E_{\text{OB}} = 178.68 \pm 0.02 \text{ mV}$

v_1 ml	v_2 ml	v_3 ml	E_B mV	H mM	h mM	E_{OB} mV	$E_j + \delta$ mV	Z
0.00	0.00		254.05	19.07		178.67	0.37	
3.00	3.00		247.32	14.63		178.66	0.28	
6.00	6.00		240.22	11.05		178.70	0.21	
9.00	9.00		232.32	8.11		178.70	0.15	
12.00	12.00		223.12	5.64	5.66		0.10	0.02
15.00	15.00		211.40	3.55	3.58		0.06	0.03
18.00	18.00		193.76	1.75	1.80		0.03	0.05
20.00		2.00	189.62	1.47	1.53			0.06
22.00		4.00	185.14	1.22	1.29			0.064
24.00		6.00	180.30	0.986	1.06			0.077
26.00		8.00	175.00	0.771	0.866			0.093
28.00		10.00	169.15	0.573	0.690			0.115
30.00		12.00	162.86	0.388	0.540			0.149
32.00		14.00	156.27	0.216	0.418			0.199
34.00		16.00	150.08	0.057	0.328			0.266
36.00		18.00	144.69	-0.092	0.266			0.352
38.00		20.00	140.17	-0.231	0.223			0.446

Table 3. Survey of measurements.

$B = 5.08_5 \times 10^{-4} \text{ M}$		$B = 1.017 \times 10^{-3} \text{ M}$			$B = 1.001 \times 10^{-2} \text{ M}$			
$-\log h$	η	$-\log h$	η	Z	$-\log h$	Z	$-\log h$	η
2.740	0.015	2.447	0.004	0.03	2.403	0.005	2.680	0.011
2.886	0.019	2.746	0.013	0.05	2.646	0.030	2.924	0.046
3.048	0.027	2.817	0.015	0.06	2.907	0.102	3.086	0.097
3.176	0.031	2.892	0.020	0.064	3.066	0.222	3.131	0.123
3.279	0.048	2.974	0.025	0.077	3.119	0.286	3.167	0.149
3.291	0.051	3.063	0.029	0.093	3.161	0.349	3.215	0.177
3.375	0.061	3.161	0.040	0.115	3.201	0.410	3.244	0.210
3.470	0.081	3.268	0.058	0.149	3.237	0.468	3.276	0.239
3.573	0.113	3.378	0.084	0.199	3.292	0.580	3.301	0.265
3.686	0.152	3.481	0.115	0.266	3.347	0.684	3.328	0.284
3.783	0.223	3.572	0.157	0.352	3.398	0.779	3.357	0.321
3.857	0.259	3.646	0.201	0.446	3.444	0.866	3.387	0.349
							3.409	0.374
							3.432	0.401

Table 3 (continued).

$B = 2.50_0 \times 10^{-2} \text{ M}$			$B = 5.00_2 \times 10^{-2} \text{ M}$			$B = 1.000 \times 10^{-1} \text{ M}$		
$-\log h$	η	Z	$-\log h$	η	Z	$-\log h$	η	Z
2.258	0.004	0.004	2.281	0.012	0.025	1.914	0.003	0.014
2.380	0.004	0.012	2.462	0.023	0.058	2.112	0.007	0.027
2.520	0.013	0.025	2.527	0.031	0.079	2.290	0.018	0.057
2.629	0.025	0.053	2.585	0.037	0.102	2.400	0.035	0.089
2.710	0.039	0.089	2.620	0.050	0.127	2.467	0.051	0.126
2.780	0.055	0.128	2.660	0.057	0.150	2.549	0.082	0.200
2.821	0.072	0.168	2.682	0.070	0.174	2.606	0.110	0.252
2.860	0.088	0.208	2.706	0.077	0.197	2.646	0.136	0.303
2.889	0.104	0.246	2.750	0.095	0.240	2.672	0.142	0.324
2.924	0.120	0.283	2.801	0.122	0.299	2.678	0.157	0.346
2.947	0.136	0.320	2.814	0.131	0.318	2.703	0.173	0.384
2.975	0.149	0.353	2.836	0.146	0.353	2.726	0.193	0.418
2.991	0.166	0.387	2.850	0.158	0.405	2.752	0.206	0.447
3.017	0.181	0.418	2.873	0.178	0.417	2.782	0.234	0.498
3.031	0.195	0.449	2.910	0.207	0.472	2.815	0.257	0.535
3.039	0.210	0.479	2.939	0.232	0.521			
3.081	0.238	0.533	2.971	0.256	0.564			
3.123	0.279	0.609						
3.161	0.319	0.678						
3.192	0.356	0.738						
3.221	0.392	0.793						
3.246	0.421	0.843						

Data

Tables 1 and 2 show two representative series of measurements in detail. For each point the following quantities have been computed: H , h , $Z = (h - H) B^{-1}$, $[\text{In}^{3+}] = b$, $\eta = \log(B/b)$ and in the first part of each titration E_0 .

As the base NaHCO_3 solutions were used following Hedström [14] since NaOH is unsuitable because a local excess of OH^- might cause an irreversible precipitation. After the addition of NaHCO_3 a small turbidity sometime appeared but the precipitate redissolved within a few minutes. Since the pH in all solutions was lower than 4, the hydrolysis of H_2CO_3 was negligible. Moreover the CO_2 concentration was always very low, since a strong stream of N_2 passed through the solution.

All the data are given in a compact form in Figs. 1 and 2 and in Table 3. Fig. 1 shows the family of functions $Z = Z(\log h)_B$, Fig. 2 $\eta = \eta(\log h)_B$. The mathematical analysis of these functions will be the starting point for finding the mechanism of the hydrolysis process.

The composition of the hydrolysis products and the reaction mechanism

To evaluate the experimental data, the systematic approach developed by Sillén [6, 7] will be closely followed.

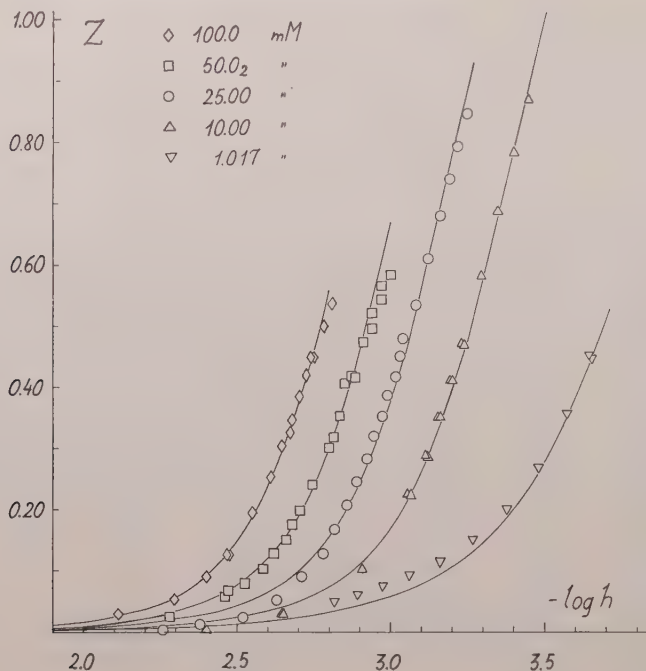


Fig. 1. Z , the average number of OH bound per indium ion, as a function of $\log h$. Curves are calculated with $p\kappa_1=4.42$, $p\kappa_2=8.3$ and hypothesis III a, $k_0=0.3$, $\log k=-4.69$.

In the first instance, only the data for the concentration range $B=10-100$ mM will be discussed. It may be observed that within the limits of experimental error the points $Z(\log h)_B$ shown in Fig. 1 and $\eta(\log h)_B$ in Fig. 2 form parallel curves and the difference quotients

$$\left(\frac{\Delta \log B}{\Delta \log h}\right)_Z = \left(\frac{\Delta \log B}{\Delta \log h}\right)_\eta = 2. \quad (4)$$

Using the arguments given in [6], (4) implies that the predominant species formed by hydrolysis have the general composition $\text{In}((\text{OH})_2\text{In})_n^{(3+n)+}$.

In order to test the validity of (4) the new variable $x = \log B - 2 \log h$ is introduced. Then assuming (4) $Z(\log h, B)$ and $\eta(\log h, B)$ are transformed to $Z(x)$ and $\eta(x)$; so that they become functions of the single variable x .

When $Z(x)$ and $\eta(x)$ were plotted, points obtained from measurements with $B=50$ and 100 mM formed a single curve in accordance with (4) but the data for lower values of B showed small positive deviations definitely exceeding the experimental inaccuracy, and these deviations increased with decreasing B .

To explain the data for low values of B it may be assumed that besides the main products, $\text{In}((\text{OH})_2\text{In})_n^{(3+n)+}$, mononuclear species are also formed in appreciable concentrations.

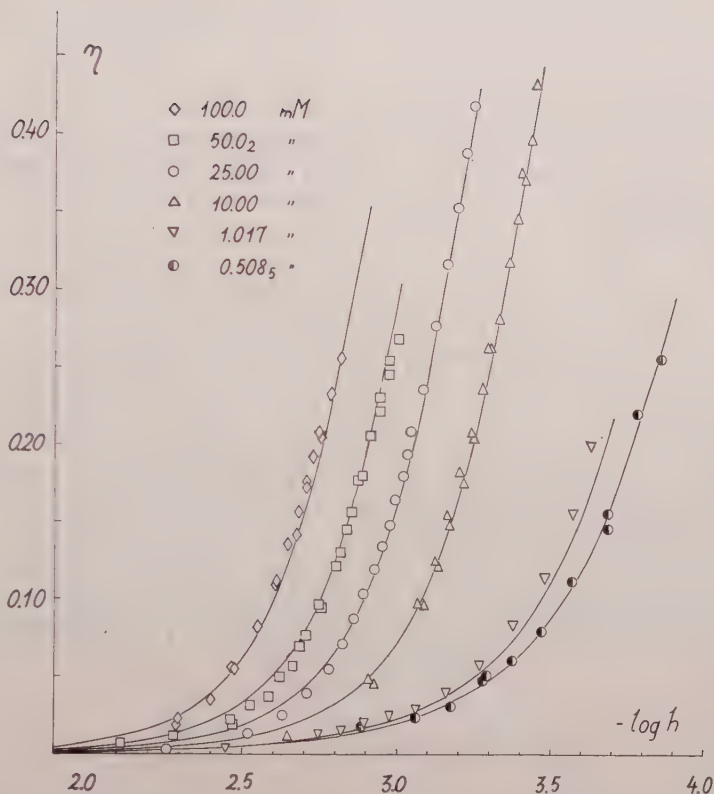


Figure 2. $\eta = \log \frac{[\text{In (III)}]_{\text{total}}}{[\text{In}^{3+}]}$ as a function of $\log h$. Curves are calculated with the same constants as in Fig. 1.

In order to find both the polynuclear and mononuclear species it was assumed as a *first approximation* that mononuclear products only are formed at the lowest value of B investigated (0.5 mM). Thus at $B = 0.5 \text{ mM}$

$$B = b + [\text{In OH}^{2+}] + [\text{In (OH)}_2^+] + \dots \quad (5)$$

Introducing the formation constants

$$\kappa_1 = [\text{In OH}^{2+}] b^{-1} h, \quad \kappa_2 = [\text{In (OH)}_2^+] b^{-1} h^2 \quad (6)$$

we obtain from (5)

$$\left(\frac{B}{b} - 1\right) h \equiv \varphi h = \kappa_1 + \kappa_2 h^{-1} + \dots \quad (7)$$

Plotting φh against h^{-1} , the experimental points for the amalgam measurements gave with a good approximation a straight line indicating that only In OH^{2+} and

$\text{In}(\text{OH})_2^+$ are present in appreciable amounts. The intercept gave $\kappa_1 = 4 \times 10^{-5} \text{ M}$ and the slope $\kappa_2 = 1 \times 10^{-8} \text{ M}^2$, which are thus a first approximation of the mononuclear constants.

The main problem of determining the formation constants of the polynuclear ions will now be reconsidered. From the experimental data for b and h and the preliminary values of κ_1 and κ_2 we can subtract the mononuclear complexes and "corrected" functions B_c , η_c , and Z_c corresponding to those analyzed by Sillén [6, 7] can be obtained.

$$B_c = B - b(\kappa_1 h^{-1} + \kappa_2 h^{-2}) = b + \sum_p \sum_{q=1} q [\text{B}_q(\text{OH})_p] \quad (8)$$

$$B_c Z_c = BZ - b(\kappa_1 h^{-1} + 2\kappa_2 h^{-2}) = \sum_p \sum_{q=1} p [\text{B}_q(\text{OH})_p] \quad (9)$$

$$\eta_c = \log (B_c b^{-1}) \quad (10)$$

A plot of Z_c and η_c against $x_c = \log B_c - 2 \log h$ gave single curves $Z_c(x_c)$ and $\eta_c(x_c)$, confirming the previous conclusion that the main polynuclear products are of the form $\text{In}((\text{OH})_2\text{In})_n^{(3+n)+}$. Hence

$$\eta_c = \log (1 + \sum (n+1) k_n u^n) \quad (11)$$

$$Z_c = \frac{2 \sum n k_n u^n}{1 + \sum (n+1) k_n u^n} \quad (12)$$

$$10^{x_c} = B_c h^{-2} = u (1 + \sum (n+1) k_n u^n) \quad (13)$$

where k_n is the constant of formation of the n th complex and

$$u = b h^{-2}. \quad (14)$$

These formulas explain the basis of our previous argument: thus η_c and Z_c must be functions of the single variable x_c since all three quantities are functions of u only.

To decide whether one species predominates or several are formed, the experimental data $Z_c(x_c)$ and $\eta_c(x_c)$ have been superimposed on the families of curves given by Sillén [7] assuming different reaction mechanisms.

No reasonable agreement could be obtained in the whole x_c range with curves calculated assuming that only one complex is formed ("hypothesis II"). Moreover, the number of OH bound per complex should be a constant when a single complex, $\text{In}((\text{OH})_2\text{In})_n$, is formed. This quantity $B_c Z_c (B_c - b)^{-1}$ was calculated from the data and was found to increase continuously in the whole range of x_c indicating the formation of complexes with increasing values of n .

Next the data were compared with the family of curves calculated assuming a repeated mechanism ("hypothesis IIIa"). According to this hypothesis n may have an unlimited series of values and the formation constant of the n th complex, k_n , is given by $k_n = k_0 k^n$, where k_0 and k are constants. A satisfactory fit was obtained with k_0 values lying between 0.1 and 0.5. A number of functions $Z_c(X_c)$ and $\eta_c(X_c)$, $X_c = x_c + \log k$, was now calculated with $k_0 = 0.1 - 0.5$; the best

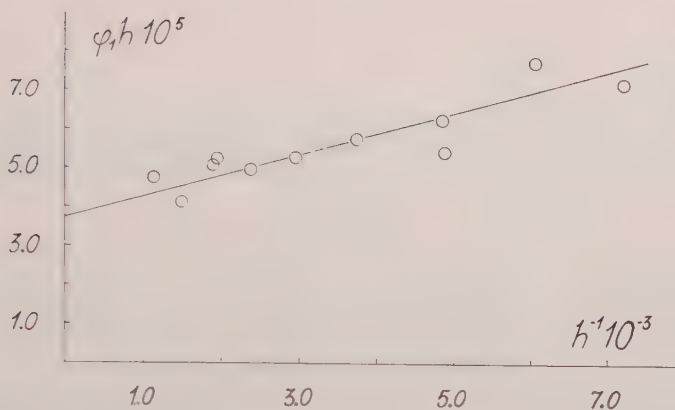


Fig. 3. Determination of z_1 and $z_2 \cdot \varphi_1 h$ (equation (15)) as a function of h^{-1} . The line represents the equation $\varphi_1 h = 3.8 \times 10^{-5} + 5 \times 10^{-9} h^{-1}$.

fit was found with $k_0 = 0.3$. From the difference $X_c - x_c$ the $Z_c(x_c)$ plot gave $\log k = -4.69$, the $\eta_c(x_c)$ plot -4.70 .

As a *second approximation*, B_c (this correction is rather small compared with $B - b$) was calculated for $B = 0.5085 \text{ mM}$ with $k_0 = 0.3$ and $\log k = -4.70$ and

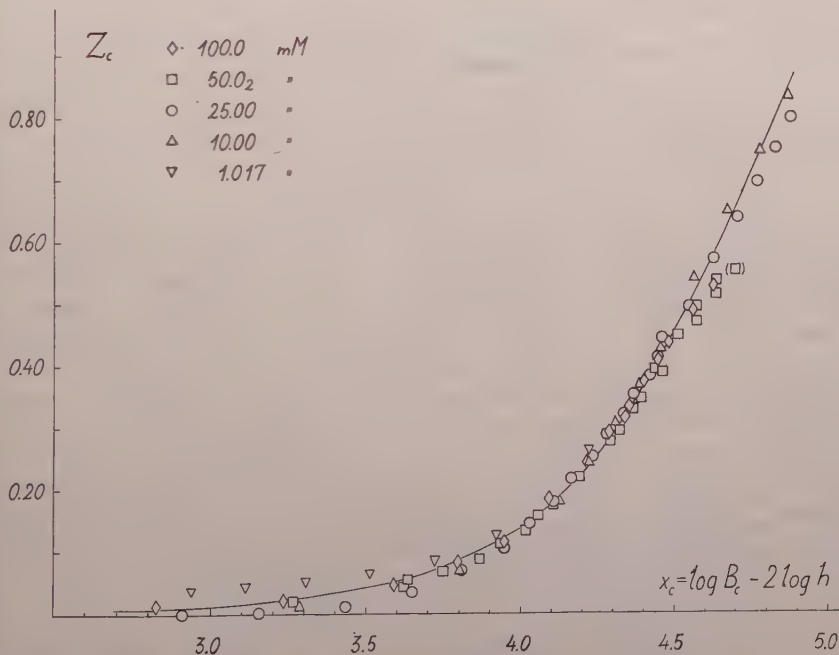


Fig. 4. Z_c (equation (12)) as a function of x_c (equation (13)). The curve is calculated with hypothesis III a $k_0 = 0.3$, $\log k = -4.69$.

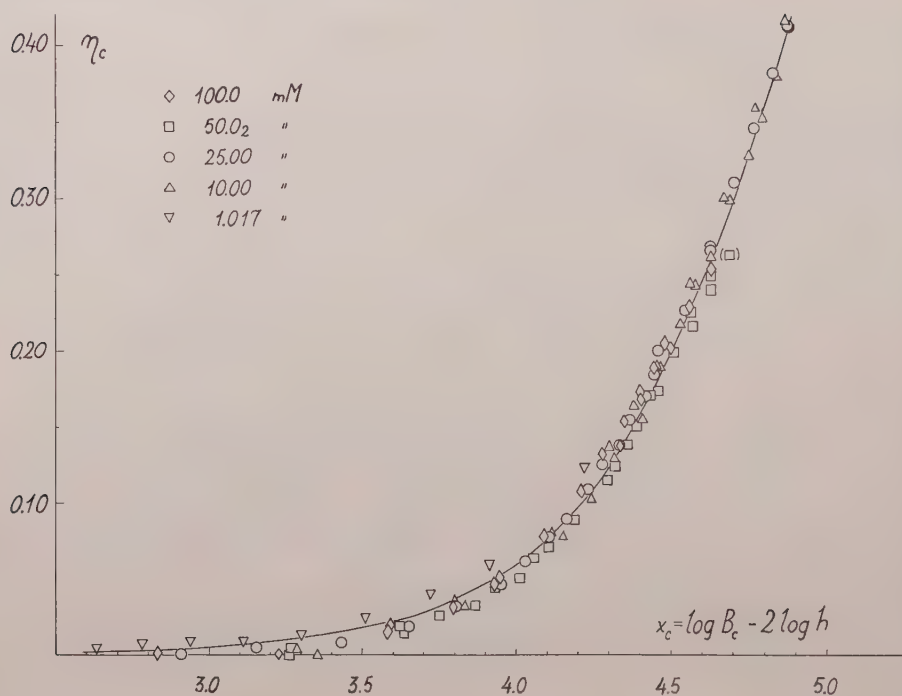


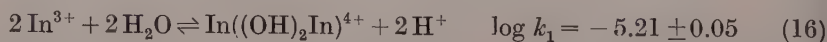
Fig. 5. η_c (equation (11)) as a function of x_c (equation (13)). The curve is calculated with hypothesis III a $k_0 = 0.3$, $\log k = -4.69$.

$$\frac{(B - B_0)h}{b} = \varphi_1 h = \kappa_1 + \kappa_2 h^{-1} \quad (15)$$

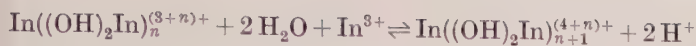
was plotted as a function of h^{-1} , cf. Fig. 3. From the intercept and slope of the line the following constants were estimated:

$$\begin{aligned} p \kappa_1 &= p k_{a1} = 4.42 \pm 0.05 \\ p \kappa_2 &= 8.3 \pm 0.1 \\ p k_{a2} &= p \kappa_2 - p \kappa_1 = 3.9 \pm 0.2. \end{aligned}$$

With these values of κ_1 and κ_2 , $\eta_c(x_c)$ and $Z_c(x_c)$ were again computed (Figs. 4 and 5); no significant change from the first approximation was introduced for $B \geq 10$ mM but the agreement of the data for $B = 1$ mM was considerably improved. The most probable values for the first step are therefore



and for all consecutive steps



$$\log k = -4.69 \pm 0.04. \quad (17)$$

The curves $Z(\log h)_B$ and $\eta(\log h)_B$ are given in Figs. 1 and 2 calculated with the final mononuclear constants and with the constants of (16) and (17); they show a satisfactory agreement with the experimental data.

Direct analysis

It has been demonstrated by Sillén [7] that the power series $g(u) = \sum k_n u^n$ can be obtained directly from the experimental data without making any assumption concerning the dependence of k_n on n , provided that the general composition of the reaction products is known. Since there is strong evidence that the polynuclear ions have the general formula $\text{In}((\text{OH})_2\text{In})_n^{(3+n)+}$ equations (14), (15) and (16) of [7] may be written in the form

$$\log(1+g) = \int_{-\infty}^{x_c} \frac{Z_c}{2} dx_c + \log\left(1 - \frac{Z_c}{2}\right) + \frac{Z_c}{2} \log e \quad (18)$$

$$\log u = x_c - \frac{Z_c}{2} \log e - \int_{-\infty}^{x_c} \frac{Z_c}{2} dx_c \quad (19)$$

$$1+g = \frac{1}{u} \int_0^u \frac{B_c}{b} du. \quad (20)$$

Since the $Z_c(x_c)$ and $\eta_c(x_c)$ values for these calculations were taken only from the concentration interval $B=10-100$ mM, it made no appreciable difference whether the final or preliminary values of κ_1 and κ_2 were used to calculate Z_c , B_c and x_c .

To apply (18) and (19), a smooth curve was drawn through the experimental $Z_c(x_c)$ points. The integration was made graphically using the trapezoid formula. More elaborate integration methods gave the same results within the limits of experimental uncertainty.

To compute $g(u)$ from the data obtained with the amalgams, a smooth curve was drawn through the experimental points $\eta_c(x_c)$, then η_c was read off at a number of values of x_c , and the corresponding values of u were calculated from $\log u = x_c - \eta_c$. The integration (20) was made using the trapezoid formula.

The values of $g(u)$ computed from both sets of data are given in fig. 6. Calculating the average number of n , $\bar{n} = d \log g / d \log u$, it was found to start at 1.0 and to increase continuously in the whole investigated range of u , attaining the maximum value 2.0. Since the slope of the $\bar{n}(\log u)$ curve shows no decrease as n attains two, complexes with at least $n=3$ must also be present in appreciable amounts at the highest values of u studied. If only the first two complexes with $n=1$ and 2 had been formed, $d\bar{n}/d \log u$ would tend to zero as $\bar{n} \rightarrow 2$.

It would not be difficult to calculate k_n values to explain the experimental $g(u)$ function, e.g. by the method of least squares or by successive extrapolations. With these methods the experimental uncertainty does not permit the calculation

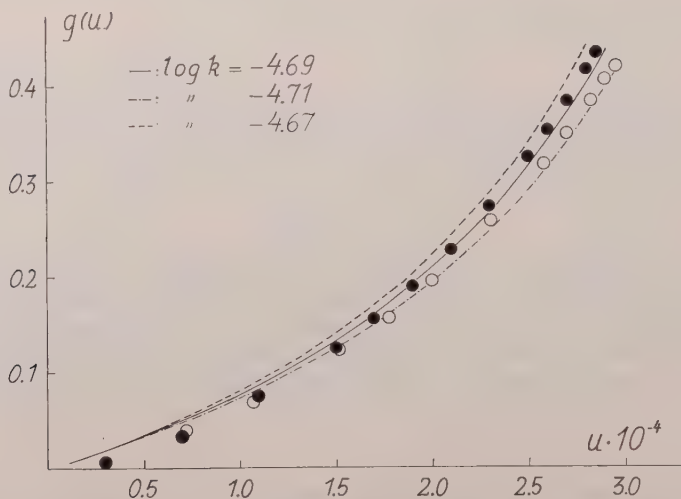


Fig. 6. $g(u)$ as a function of u . ●: amalgam electrode measurements; ○: glass or quinhydrone electrode measurements. Curves are calculated with "hypothesis III a", $k_0 = 0.3$ and $\log k = -4.67, -4.69$ and -4.71 .

of more than two or perhaps three constants, but there seems to be no plausible reason for assuming that the maximum value of n is, say, three. It is believed that methods of evaluation in which no limit is set a priori to n are definitely to be preferred.

Direct analysis affords a check on the repeated mechanism ("hypothesis III a") proposed in the preceding section. In Fig. 6 the $g(u)$ curves shown are calculated using hypothesis III a with $k_0 = 0.3$ and $\log k = -4.67, -4.69$ and -4.71 . The agreement between the curves and the $g(u)$ values calculated by direct analysis is very satisfactory except for points of lowest values of u where the integration is inevitably uncertain. Hypothesis III a must thus be considered to be a very satisfactory approximation to the experimental data.

It might be helpful to visualize the results of the computations by giving the distribution of the complexes as a function of $\log k$ for three different values of B , cf. Fig. 7.

Structure of the hydrolysis products

Emf data alone cannot give information concerning the structure of the ions formed by hydrolysis. Consideration of the crystal structure of the basic salts has often proved to be of value in predicting the probable structure of the hydrolysis products. Forsberg [15] has investigated the crystal structure of indium oxyhalides in this laboratory and has found that these crystals contain sheets of $(\text{InO})_n$ separated by the halogens. The ions $\text{In}((\text{OH})_2\text{In})_n^{(3+n)+}$ or perhaps better $\text{In}(\text{OIn})_n^{(3+n)+}$ may be fragments of the sheets present in the basic salts. As hydrolysis proceeds these fragments attain a critical size and precipitation sets in.

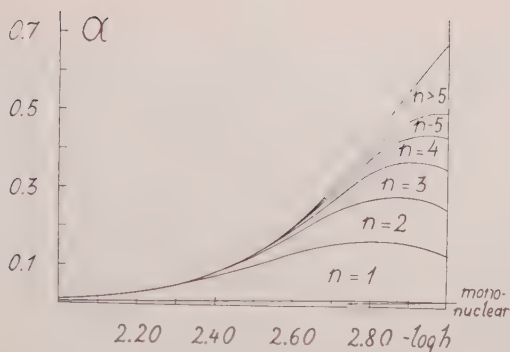


Fig. 7 a.

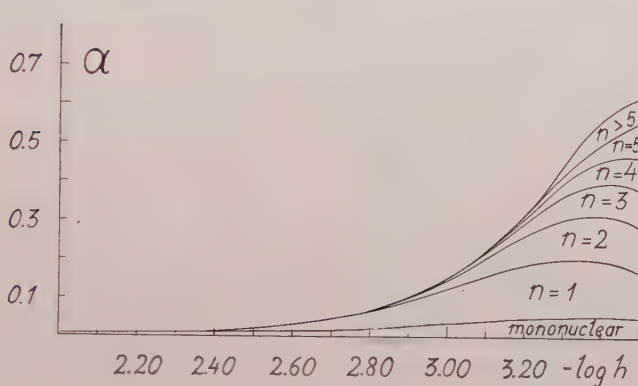


Fig. 7 b.

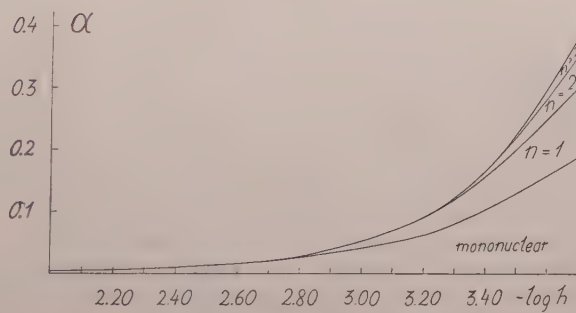


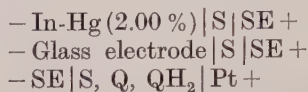
Fig. 7 c.

Fig. 7. Distribution of In(III) between different complexes. The abscissa is $-\log h$, the ordinate is $\alpha = [\text{In(III)}]_{\text{present as complex ion}} / [\text{In(III)}]_{\text{total}}$. If a vertical line is drawn at a $\log h$ value the segment of the line falling in the zone corresponding e.g. to $\text{In}((\text{OH})_2\text{In})_2^{5+}$, represents the fraction of $[\text{In(III)}]_{\text{total}}$ present as that ion. The $\alpha(\log h)_B$ curves have been calculated assuming "hypothesis III a", $k_0 = 0.3$, $\log k = -4.69$; $p x_1 = 4.42$, $p x_2 = 8.3$.

Fig. 7 a $[\text{In(III)}]_{\text{total}} = 100 \text{ mM}$, Fig. 7 b $[\text{In(III)}]_{\text{total}} = 10 \text{ mM}$, Fig. 7 c $[\text{In(III)}]_{\text{total}} = 1 \text{ mM}$.

Summary

The hydrolysis equilibria of the In(III) ion have been investigated at 25°C using the cells



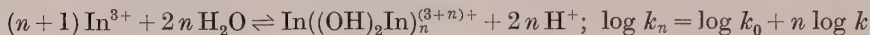
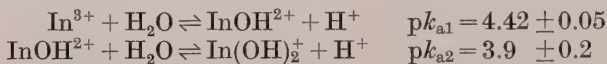
SE denotes the half-cell $\text{Ag, AgCl} | 10 \text{ mM NaCl, } 2990 \text{ mM NaClO}_4 | 3 \text{ M NaClO}_4$, and the solution S had the general composition



where H is the analytical hydrogen ion concentration assuming no hydrolysis.

The total indium concentration B varied between 0.5085 and 100 mM and the equilibrium concentration of hydrogen ion from values where the hydrolysis is negligible until a precipitate was formed.

The data can be explained assuming the equilibria



where n assumes a series of values and $k_0 = 0.3$, $\log k = -4.69 \pm 0.04$.

The polynuclear complexes $\text{In}((\text{OH})_2\text{In})_n^{(3+n)+}$ or $\text{In}(\text{OIn})_n^{(3+n)+}$ predominate over the mononuclear ones at the higher In concentrations studied (see Fig. 7).

An independent method of calculation (direct analysis) indicates that the assumption $k_n = k_0 k^n$ ("hypothesis III a") is quite a good approximation to the experimental data.

ACKNOWLEDGEMENTS

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I am indebted to Dr. Francis J. C. Rossotti for correcting the English text.

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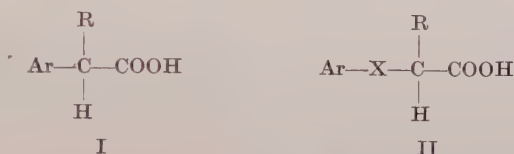


Stereochemical studies. I

On the synthesis and resolution of α -(2-Naphthyl)-propionic acid

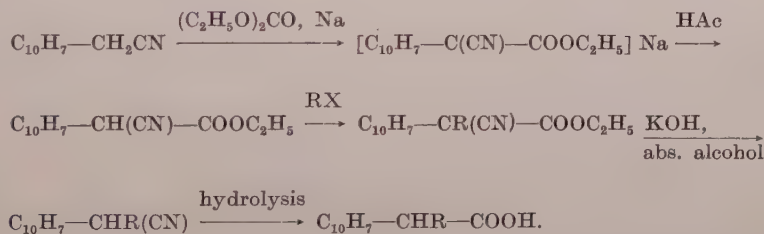
By BERNDT SJÖBERG

In connection with stereochemical work at this Institute, the author has prepared and resolved α -(2-naphthyl)-propionic acid for comparative studies of the rotatory dispersion of carboxylic acids of the general structure I and II, where X might be $(CH_2)_n$, O, S, Se, Te or NH.



In 1948 Wilds and Meader (1) reported a method for the synthesis of α -(2-naphthyl)-propionic acid using diazoethane in the Arndt-Eistert synthesis on 2-naphthoylechloride. However, as this method seems to be rather timeconsuming, the author chose an alternative way.

In 1947 Wideqvist (2) reported a simple method of obtaining α -naphthylated acids according to:



In the present investigation α -(2-naphthyl)-propionic acid was prepared according to the latter description (2).

The resolution of the racemic acid could be performed with cinchonidine. Recrystallization of the cinchopidine salt from pure methanol gave the (+)-antipode (measured in abs. alcohol) and from dilute ethanol the (—)-form.

The growth-regulating activity of the acid is under investigation.

Experimental

The conversion of 2-methylnaphthalene into 2-bromomethyl-naphthalene and of the latter into 2-naphthylacetonitrile has been performed in the usual way (3, 4).

Ethyl 2-naphthylcyanoacetate.—16.7 g (0.1 mole) of 2-naphthylacetonitrile, 60 ml of diethyl carbonate and 2.3 g of sodium were placed in a flask, fitted with a reflux condenser. The flask was heated in an oil bath to about 110°, and thereby a vigorous reaction began. When the sodium had dissolved, the reflux condenser was changed for a short Vigreux column and the alcohol produced by the reaction was distilled off. 6.2 ml of alcohol was obtained (calc. 5.8 ml). After cooling, the reaction mixture was acidified with acetic acid, washed free of acid, dried and fractionated. 17 g (72 %) of a viscous, colourless oil was obtained, boiling at 185–186°/2mm Hg. For identification, a small part of the oil was converted into the amide. The amide, when recrystallized from dilute alcohol, gave microscopic needles with m.p. 182–183°.

Analysis of amide: Subst. 19.62 mg; CO₂ 53.27 mg; H₂O 8.24 mg

C₁₃H₁₀N₂O Calc. C 74.25 H 4.80

Found C 74.10 H 4.70

Ethyl α-(2-naphthyl)-cyanopropionate.—In a flask, fitted with a reflux condenser, 1.6 g of sodium was dissolved in 25 ml of abs. alcohol. To the ethylate were added 16 g of ethyl 2-naphthylcyanoacetate and 20 g of methyl iodide. The mixture was refluxed for three hours. The alcohol was distilled from the resulting neutral mixture and the residue shaken with potassium hydroxide solution to dissolve the sodium iodide and any unchanged ethyl 2-naphthylcyanoacetate. Yield of the crude product was 15 g (90 %). It was not further purified.

α-(2-Naphthyl)-propionic acid.—15 g of the crude ethyl α-(2-naphthyl)-cyanopropionate and 10 g of potassium hydroxide were dissolved in 100 ml of alcohol and the mixture was boiled under reflux for half an hour in order to decarboxylate the ester. Water was then added and the mixture refluxed for 24 hours. The alcohol was distilled off and the residue extracted with ether in order to remove unsaponified nitrile. When the water solution was acidified with hydrochloric acid, the organic acid precipitated as an amorphous powder. The acid was recrystallized from dilute alcohol and from ligroin (b.p. 85–110°). It formed small needles with m.p. 129–130°. Wilds and Meader (1) reported 129.5–130°. The yield of pure acid was 10 g (80 % calculated on ethyl α-(2-naphthyl)-cyanoacetate).

0.1112 g acid: 7.36 ml 0.0754N NaOH

C₁₃H₁₂O₂ (200.23) Calc. equiv. wt. 200.2

Found equiv. wt. 200.4

Resolution of the racemic acid.—In preliminary tests, 0.001 mole of optically inactive acid and 0.001 mole of the base were dissolved in different solvents. The crystals formed were decomposed in the usual way. The acid was extracted with ether and the optical activity measured in abs. ethanol. The results are given in Table 1.

Equimolecular quantities of the racemic acid (20.02 g, 0.1 mole) and cinchonidine (29.44 g) were dissolved in 800 ml of hot methanol and the solution was left in a refrigerator for 24 hours. The salt was filtered off, washed with cold methanol and

Table 1. Preliminary tests on the resolution of α -(2-naphthyl)-propionic acid.

Base	$[\alpha]_D^{25}$ of acid in abs. ethanol			
	From methanol	From ethanol	From acetone	From ethylacetate
Cinchonine	+ 7°	+ 19°	—	—
Cinchonidine	+ 50°	—27°	$\pm$ 0°	—
Quinine	+ 19°	+ 14°	—	—
Chinidine	$\pm$ 0°	oil	oil	oil
Brucine	oil	oil	oil	oil
Strychnine	oil	oil	oil	oil
(+)-Phenylethylamine	$\pm$ 0°	$\pm$ 0°	—	—
Morphine	oil	oil	—	oil

Table 2. Recrystallization of the cinchonidine salt of α -(2-naphthyl)-propionic acid.

Cryst. No.	Methanol (ml)	Weight of salt (g)	$[\alpha]_D^{25}$ of acid
1	800	27.9	+ 41.1°
2	600	21.8	+ 62.2°
3	500	18.3	+ 66.0°
4	500	16.6	—
5	500	14.8	+ 66.3°
6	500	12.6	+ 66.4°

Table 3. Recrystallization of the cinchonidine salt of α -(2-naphthyl)-propionic acid.

Cryst. No.	Solvent (ml)		Weight of salt (g)	$[\alpha]_D^{25}$ of acid
	Ethanol	Water		
0	—	—	27.5	— 47.2°
1	260	10	23.2	— 61.9°
2	220	8	18.6	— 66.4°
3	130	5	16.6	— 66.5°

Table 4. $[\alpha]_D^{25}$ and $[M]_D^{25}$ of (+)- α -(2-naphthyl)-propionic acid.

Solvent	mg of acid	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Benzene	114.0	+ 1.76°	+ 77.2°	+ 154.6°
2,2,4-Trimethylpentane	11.60	+ 0.19°	+ 82°	+ 164°
Acetic acid	116.8	+ 1.81°	+ 77.4°	+ 155.0°
Acetone	100.9	+ 1.98°	+ 98.1°	+ 196.4°
Chloroform	105.3	+ 1.50°	+ 71.2°	+ 142.5°
Ethanol	118.3	+ 1.63°	+ 68.8°	+ 137.7°
Water*	109.7	— 0.36°	— 16.4°	— 32.8°

* The acid neutralized with NaOH (pH 7)

dried. From a sample (about 0.3 g) the acid was isolated and the optical activity measured in abs. alcohol. The remaining salt was recrystallized from methanol according to Table 2.

The mother liquors from the two first crystallizations were combined. After evaporating the methanol, 27.5 g of the cinchonidine salt was obtained. The salt was recrystallized from dilute ethanol. The progress of the resolution was followed as described above.

(+)- α -(2-Naphthyl)-propionic acid.—The salt from cryst. No. 6 (Table 2) was decomposed with sulfuric acid and the organic acid extracted with ether. It was recrystallized once from cyclohexane and twice from ligroin (b.p. 85–110°). The yield of crude acid was 4.5 g; after the recrystallizations there remained 4.1 g. From the mother liquors No. 4 and 5 another 1.3 g of pure acid was obtained. The acid forms small needles with m.p. 139.5–141°.

0.1173 g acid: 7.71 ml 0.0754N NaOH

$C_{13}H_{12}O_2$ Equiv. wt. calc. 200.2 found 200.1

Weighed amounts of the acid were dissolved in different solvents, made up to 10.00 ml and the optical activity measured. The results are given in Table 4.

(—)- α -(2-Naphthyl)-propionic acid.—From the last fraction (Table 3) 6.6 g of crude acid was obtained. It was recrystallized once from cyclohexane and twice from ligroin (b.p. 85–110°). The yield of pure acid was 4.9 g. M.p. 139.5–141°.

0.1055 g acid: 7.02 ml 0.0754N NaOH

$C_{13}H_{12}O_2$ Equiv. wt. calc. 200.2 found 200.0

0.1080 g acid dissolved in abs. ethanol to 10.00 ml: $2\alpha_D^{25} = -1.48^\circ$ $[\alpha]_D^{25} = -68.6^\circ$; $[M]_D^{25} = -137.3^\circ$.

Summary

α -(2-Naphthyl)-propionic acid has been prepared in a new way and resolved into the optically active forms using cinchonidine. The cinchonidine salt in pure methanol gave the (+)-acid and in dilute ethanol the (—)-acid. The rotary power found was $[\alpha]_D^{25} = +68.8^\circ$ and -68.6° respectively (in abs. ethanol).

The author is greatly indebted to Professor Arne Fredga for his interest in this work and for valuable discussions.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, November 1955.

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Spectrochemical studies on disulphides

II. Isomeric dithio-dibenzoic acids and their dimethyl esters

By LENNART SCHOTTE

With 4 figures in the text

At this institute spectrochemical studies are in progress on disulphides and diselenides with special emphasis on carboxylic substituted derivatives (1, 2). Our investigations have hitherto been concerned with non-aromatic compounds but it seemed of value to get some information even on diaryldisulphides. The spectral behaviour as a function of substituent influence could then be studied for the isomers. It should be pointed out, however, that the ways of approaching the problem are somewhat different for the aromatic derivatives on one side and the non-aromatic on the other. Nevertheless, regardless of the approach taken information about the character of the disulphide linkage will be obtained.

The three isomers of dithio-dibenzoic acid and the corresponding dimethyl esters have now been investigated as concerns ultraviolet and infrared absorption. Due to the unfavourable solubility conditions of the free acids, however, the UV examination was carried out on the di-sodium salts. The acids were prepared according to accepted methods (3, 4, 5). The somewhat tedious purification is described in the experimental part. The dimethyl esters were obtained from the acids with diazomethane; only the *o*-ester has hitherto been described in the literature (6, 7).

In Fig. 1 the UV absorption curves are shown for the isomeric dimethyl dithio-dibenzoates and compared with the spectrum of di-*o*-nitrophenyl-disulphide. The measurements were made in alcoholic solution. In general the curves are characterized by high intensities and marked differences between the isomers. The *o*-ester has two maxima, at 314 and 255 $m\mu$ resp., whereas the *m*-compound only has a slightly developed maximum at 296 $m\mu$. The absorption curve for dimethyl *p,p'*-dithio-dibenzoate shows a very broad and intense maximum at 276 $m\mu$. The di-*o*-nitrophenyl-disulphide gives an absorption curve, the outlines of which resemble those of the *o,o'*-dimethyl ester mentioned above. A marked shift towards longer wavelengths is observed with maxima at 354 and 243 $m\mu$ resp. together with an inflexion at 270 $m\mu$.

If we now consider the spectra of the sodium salts of the corresponding acids (in aqueous solution) given in Fig. 2, it seems obvious that the general appearance is rather similar to that reported for the esters. The differences imply that the intensities are somewhat lower and hypsochromic shifts are observed. Furthermore, where the maxima remain, they are less pronounced. The sodium salt of *o,o'*-dithio-dibenzoic acid thus has an absorption maximum at 296 $m\mu$ and a distinct inflexion near 250 $m\mu$. The curve of the *m*-compound passes through a point of inflexion at 295 $m\mu$, whereas the *p*-isomer shows a strong maximum at 265 $m\mu$.

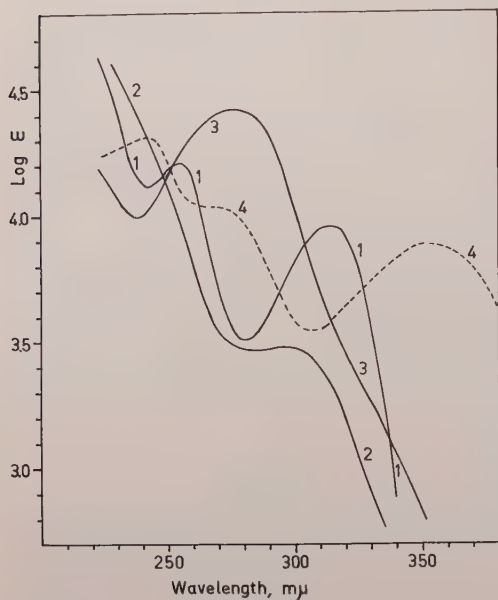


Fig. 1. UV absorption spectra in alcohol of: (1) dimethyl *o,o'*-dithio-dibenzoate; (2) dimethyl *m,m'*-dithio-dibenzoate; (3) dimethyl *p,p'*-dithio-dibenzoate; (4) di-*o*-nitrophenyl-disulphide (dashed curve).

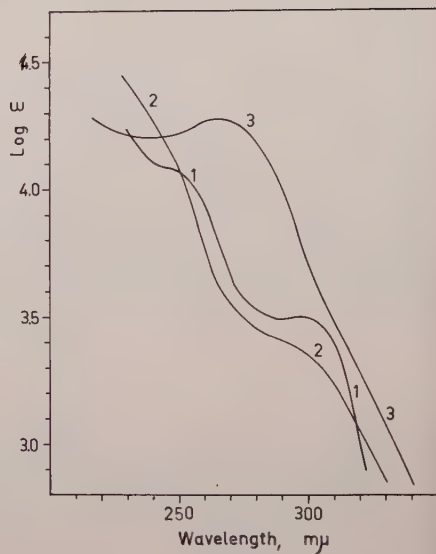
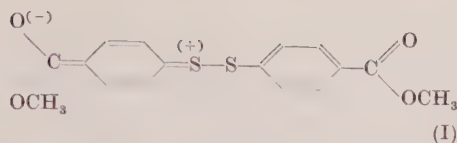


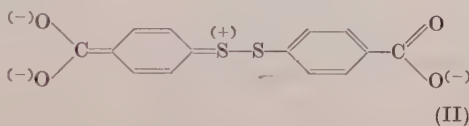
Fig. 2. UV absorption spectra in aqueous solution of the di-Na salts of: (1) *o,o'*-dithio-dibenzoic acid; (2) *m,m'*-dithio-dibenzoic acid; (3) *p,p'*-dithio-dibenzoic acid.

There are a few papers published about UV absorption spectra of some aromatic disulphides (e.g. 8, 9). From the data available it appears that diphenyl disulphide has a maximum near 240 $m\mu$ (238 $m\mu$ in (8); 241 $m\mu$ in (9)) and that the *p,p'*-dicyano derivative shows a broad top at 267–273 $m\mu$ (9). For the *p,p'*-dichloro- and *p,p'*-dibromo-diphenyl disulphides maxima at 242 and 248 $m\mu$ resp. are observed (8). Although beyond the scope of this paper it may be pointed out that alkyl sulphide substituted benzoic acids have also been investigated (10, 11). The powerful conjugation between a *p*-sulphide group and the carbethoxyl function is well established here.

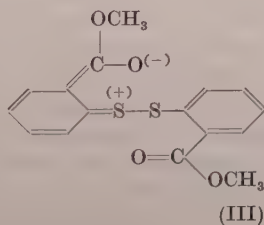
In order to interpret the spectra presented in Figs. 1 and 2 we first establish that the absorption curves for the salt of *m,m'*-dithio-dibenzoic acid and its dimethyl ester are rather similar. The slightly developed maximum for the ester at 296 $m\mu$ corresponds to the inflexion at 295 $m\mu$ with somewhat lower intensity for the salt. It seems justified to conclude then that the carboxylic function does not essentially contribute to the polar excited structures, which instead may represent states where the non-bonding *p*-electrons on sulphur conjugate, in some way, with the benzene π -electrons. It is thus already confirmed that the *m*-acid is obviously different from the other isomers as could be expected, if mesomeric effects are considered. Further discussion is best founded on the curves in Fig. 1. The strong maximum for dimethyl *p,p'*-dithio-dibenzoate is certainly explained by a comparatively stable polar excited structure like (I), where the non-bonding electron-pair of the sulphur atom conjugates through the benzenoid system with the carbethoxy group.



That would mean a considerable bathochromic shift as compared with the unsubstituted diphenyl disulphide. It does not seem likely to the author that conjugation is extended through the disulphide group into the other benzene ring, as that would imply a structure with two like charges on adjacent atoms. Furthermore this would require the benzene planes to be coplanar with the S-S linkage. Available data, on the other hand, indicate a stabilized non-planar skew configuration for linear disulphides with a relatively fix azimuthal (dihedral) angle of about 100° . Should even such an alternative mode of conjugation contribute to the electronic excitation structures, specific absorption would be expected at higher wavelengths with considerably lower intensity due to extended conjugation but reduced transition probability. If such an absorption occurs, which is not at all sure, the maximum is probably overlapped by the intense broad maximum in the $276\text{ m}\mu$ region. As the ester group has a mesomeric effect greater than that of a carboxylic anion, the smaller conjugation in the corresponding salt causes a hypsochromic shift for the specific absorption (here $11\text{ m}\mu$). The maximum intensity is lowered for the salt, which seems likely as the resonance form (II) means diminished transition probability as compared with (I) because of the two adjacent negative charges.

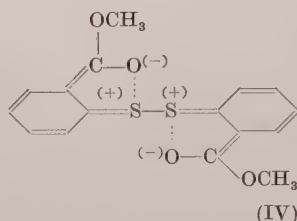


Transferring this mode of interpretation to the dimethyl *o,o'*-dithio-dibenzoate we conclude that the spectral behaviour seems characterized by a special effect, which may be called an *o*-effect. The top at $255\text{ m}\mu$ corresponds normally to the one found at $276\text{ m}\mu$ for the *p*-dimethyl ester, as the *o*-position of the ester group from steric reasons causes a decrease in intensity with accompanying short-wave displacement. The excited state resulting from high-intensity absorption of light would then contain a polar structure such as (III). A lower intensity of absorption as com-

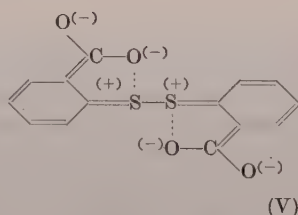


pared with (I) is expected as the steric hindrance of the *o*-substituent makes coplanar structures less energetically favoured. However, there is another absorption maximum in the *o*-ester absorption curve, at $314\text{ m}\mu$, which seems typical for the *o,o'*-

structure. It is of course difficult to give an unequivocal interpretation of this spectral detail, but as the maximum is found at higher wavelengths extended conjugation might be characteristic for the structure responsible. It is now suggested that an electronic structure such as (IV) could be justified, where interaction between adja-



cent charged atoms would give a chelate system of conjugative nature. Of course the objections mentioned above in the discussion about the *p*-isomer spectrum could be raised here, but it must then be remembered that the maximum really has lower intensity than that in the 250 $m\mu$ region and that the two like formal charges on the adjacent sulphur atoms are at least partly compensated through the electronic interaction. The structure is not energetically favoured but may be a minor contribution to the polar excited state of the molecule. Comparing the spectrum to that of the sodium salt of the corresponding acid (in Fig. 2) we note an appreciable decrease in intensity and shortwave shifts as found above for the *p*-isomer. An analogous explanation seems valid and as the difference in absorption intensity is especially high for the 300 $m\mu$ region we could expect the 255 $m\mu$ peak from the ester curve to appear only as an inflexion for the acid. Structure (V) is less favoured than the corresponding (IV).



It seems further justified to compare the dimethyl *o,o'*-dithio-dibensoate spectrum to that of di-*o*-nitrophenyl disulphide. The absorption maximum at 243 $m\mu$ corresponds surely to one for the ester in the vacuum ultraviolet region (here not detectable) and hence the point of inflexion at 270 $m\mu$ and the maximum at 354 $m\mu$ are identifiable through red-shifts of the maxima observed for the *o*-ester. The spectral interpretation is suggested to be analogous. It must be mentioned here that the outlines of the absorption curve under discussion resemble those determined by KOCH for phenyl *o*-nitrophenyl sulphide (8). The modes of spectral interpretation used are similar but the conclusions in what seems to be an analogous case are different.

The spectroscopic investigation of the isomeric dithio-dibensoic acids and their dimethyl esters includes also some studies of infrared absorption. The esters and acids have thus been examined in the potassium bromide prism region (11–24 μ).

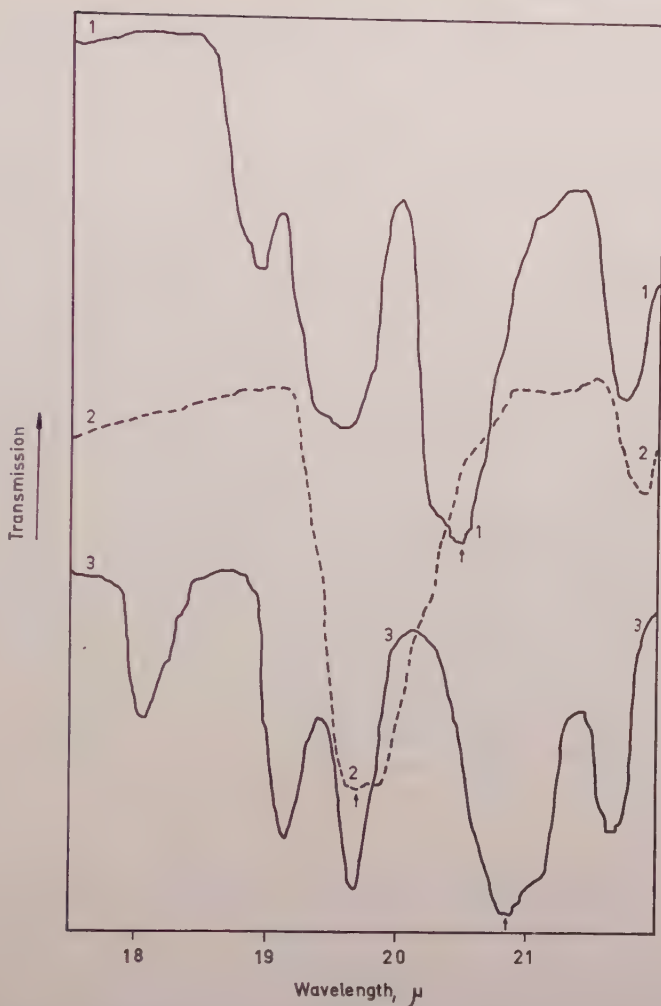


Fig. 3. IR absorption spectra between 17.5μ and 22μ of: (1) dimethyl *o,o'*-dithio-dibenzoate; (2) dimethyl *m,m'*-dithio-dibenzoate (dashed curve); (3) dimethyl *p,p'*-dithio-dibenzoate.

In general the IR bands of the disulphide group are difficult to identify but in aromatic compounds the bands appear to be more readily detectable. CYMERMAN and WILLIS (12) have found the S-S-linkage values within the range $490\text{--}430\text{ cm}^{-1}$ but they note that the band is ill-defined and difficult to identify even here. For di-*p*-cyanophenyl disulphide the absorption is found at 443 cm^{-1} and for the corresponding amide the value 461 cm^{-1} is given, whereas the unsubstituted diphenyl disulphide has its peak at 468 cm^{-1} . Considering the IR spectra of the esters and acids we conclude that an assignment of the disulphide band must be made with caution. The absorption curves are shown in the region of interest ($17.5\text{--}22\mu$) in Figs. 3 and 4. Starting with the spectrum of dimethyl *m,m'*-dithio-dibenzoate (Fig. 3:2) it seems valid to assign the broad absorption peak at about 19.8μ ($510\text{ to }503$

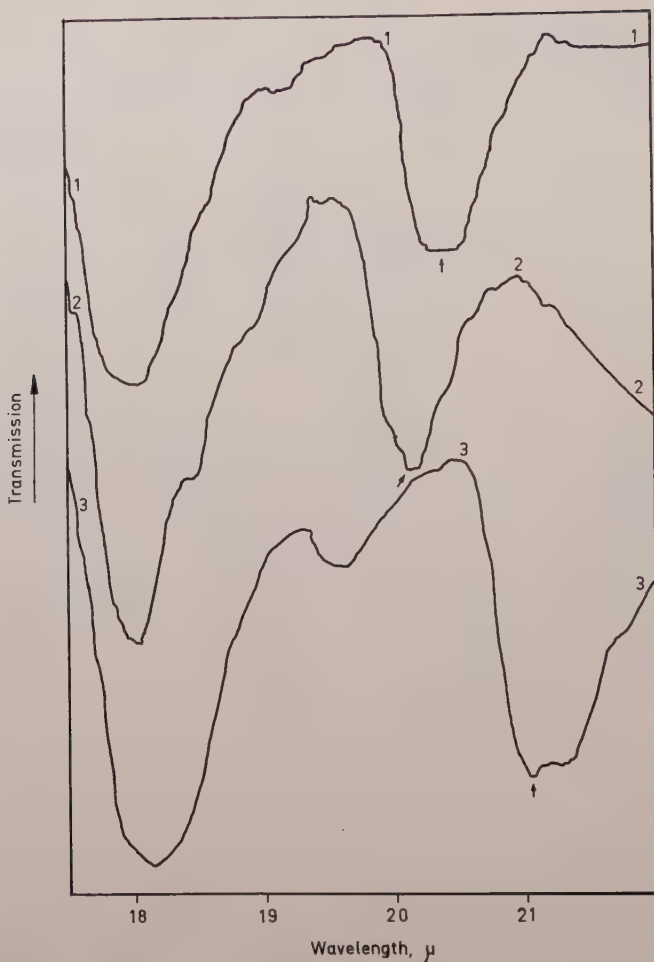


Fig. 4. IR absorption spectra between 17.5μ and 22μ of: (1) *o,o'*-dithio-dibenzoic acid; (2) *m,m'*-dithio-dibenzoic acid; (3) *p,p'*-dithio-dibenzoic acid.

cm^{-1}) to the stretching vibration of the S-S-linkage. By analogy, the value for the corresponding acid (Fig. 4:2) is found at 20.15μ (496 cm^{-1}). A comparison of the isomeric acids in Fig. 4 then allows conclusions to be drawn. The *o*-acid has its absorption peak around 20.4μ (493 to 488 cm^{-1}) and the *p*-isomer a broad maximum around 21.2μ (475 to 468 cm^{-1}), which probably consists of two peaks at 21.1μ and 21.3μ resp. The expected displacement towards longer wavelengths is evident. It is now also possible to assign the bands for the *o*- and *p*-ester, although with a little uncertainty as concerns the *o*-isomer. The stretching vibration frequency of the S-S-bond is then found at 20.85μ (480 cm^{-1}) for dimethyl *p,p'*-dithio-dibenzoate and at 20.5μ (488 cm^{-1}) for the *o*-ester. The uncertainty here involves the peak at 19.6μ (510 cm^{-1}) but the above mentioned frequency seems more likely in comparison with the value for the *o*-acid. The general tendency from the preceding discussion is

Table 1. The infrared absorption bands of the isomers of dimethyl dithio-dibensoates and di-*o*-nitrophenyl-disulphide (11–24 μ).

b = broad; vs = very strong; s = strong; m = medium; w = weak; sh = shoulder.

Dimethyl- <i>o,o'</i> -dithio-dibensoate			Dimethyl- <i>m,m'</i> -dithio-dibensoate			Dimethyl- <i>p,p'</i> -dithio-dibensoate			Di- <i>o</i> -nitrophenyl-disulphide		
Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band
11.50	870	w	11.20	893	s	11.75	851	vs	11.73	853	s
12.10	826	vs	11.94	838	vs	11.87	842	vs	12.8	781	sh
12.58	795	m	12.42	805	m	12.09	827	vs	12.86	778	s
c. 13.5	c. 741	vs b	c. 13.7	c. 730	vs b	12.85	778	w	c. 13.7	c. 730	vs b
14.23	703	s	14.90	671	vs	13.15	760	vs	14.10	709	vs
14.63	684	s	15.20	658	s	13.30	752	vs	14.77	677	m
15.34	652	s	17.05	587	w	13.95	717	w	15.31	653	s
18.92	529	w	c. 19.8	510–503	s b	14.55	687	vs	18.0	556	w b
c. 19.6	c. 510	m b	21.90	457	w	15.86	631	m	20.4	490	sh
20.5	488	s b	23.9	418	m	18.05	554	m	20.60	485	m b
21.75	460	m				19.15	522	m	21.50	465	w b
23.9	418	m				19.68	508	s	23.8	420	w b
						20.85	480	s			
						21.0	476	sh			
						21.65	462	m			
						23.9	418	w			

Table 2. The infrared absorption bands of the isomers of dithio-dibensoic acids (11–24 μ). For abbreviations see Table 1.

<i>o,o'</i> -Dithio-dibensoic acid			<i>m,m'</i> -Dithio-dibensoic acid			<i>p,p'</i> -Dithio-dibensoic acid		
Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band
12.37	808	m	11.19	894	s	11.97	848	s
12.60	794	m	11.82	846	m	12.0	833	sh
13.53	739	vs	12.32	812	s	12.25	816	s
14.35	697	s	13.43	745	vs	13.20	758	vs
15.34	652	s	14.01	714	vs	14.20	704	m
18.0	556	s b	14.86	673	vs	14.67	682	m
c. 20.4	493–488	m b	15.30	654	s	15.90	629	w
			17.05	587	w	18.2	549	s b
			18.05	554	s	19.6	510	w b
			18.45	542	sh	c. 21.2	475–468	m b
			20.15	496	m			

recognized. The spectrum of di-*o*-nitrophenyl disulphide has also been recorded but the curve is omitted here as it did not allow any reliable conclusions to be drawn concerning the S-S stretching frequency. A comparison to the *o*-ester, however, suggests an uncertain value of 20.60μ (485 cm^{-1}). The wavelengths of the absorption peaks are given in Table 1 together with those for the esters within the potassium bromide prism region. The corresponding values for the acids are found in Table 2.

Finally it should be pointed out that a clear assignment of the carbon-sulphur stretching vibration for aromatic sulphur compounds is practically impossible, as the benzene ring itself has a strong band in the region of interest ($600\text{--}700 \text{ cm}^{-1}$). It is the ν_4 -frequency, which appears in benzene as a strong band at 671 cm^{-1} (13), which persists in a large number of benzene derivatives (except *p*-disubstituted) as a band near 700 cm^{-1} . This is also valid for the isomers studied in this paper and so only the behaviour of the disulphide linkage has been discussed.

All the isomeric acids show strong absorption bands near $18 \text{ m}\mu$, which seems characteristic for some structural vibration and is also found for di-*p*-cyanophenyl disulphide (12).

Experimental

For the *ultraviolet absorption determinations* a Beckman model DU quartz spectrophotometer was used. The sodium salts were measured in water and the other compounds in alcohol as a solvent. The salts were obtained by dissolving the acids in slight excess of aqueous sodium bicarbonate solution. It was demonstrated that no dismutation of the disulphides occurred under the conditions employed.

A Perkin Elmer model 21 spectrophotometer equipped with potassium bromide prism was used for the *infrared absorption measurements*. The substances were examined as solid pressed potassium bromide plates (14). The calibration of the absorption curves is correct to $\pm 0.04 \mu$.

o,o'-Dithio-dibensoic acid was prepared from diazotised anthranilic acid and sodium disulphide (3). The purification of the isomeric dithio-dibensoic acids is difficult owing to their very low solubility. Of course the acid can be carefully precipitated from an aqueous solution of its salt, but this does not always give satisfactory results. The *o*-disulphide was purified in two ways. One part was reduced to thiosalicyclic acid in reaction with zinc dust and acetic acid and the resulting *o*-mercapto bensoic acid was repeatedly recrystallized from alcohol-water (m.p. 164°). The disulphide was then obtained from this acid by careful oxidation with iodine. Another part of the crude acid was extracted continuously with acetone, from which a pure substance deposited. This procedure was repeated twice. M.p. $292^\circ\text{--}294^\circ$.—The equivalent weight was determined by titration with $0.0685N$ NaOH. Due to the ease with which aromatic disulphides undergo dismutation, it is necessary to use an indicator with a pH range near pH 7, e.g. bromothymol blue (pH 6.0–7.6) (cf. 4, 5, 15, 16, and 17). Phenolphthalein (pH range 8.3–10) gives too low values.

Anal. Subst., 42.64 mg; 4.08 ml of $0.0685N$ NaOH (bromothymol blue). Equ. wt. Calc. 153.1; Found 152.6.

Dimethyl o,o'-dithio-dibensoate was prepared from the *o*-acid and diazomethane (18) in ether and recrystallized from methanol. M.p. $130^\circ\text{--}131^\circ$ (cf. 6 and 7).

Anal. Subst., 28.37 mg; 4.60 ml of $0.0733N$ NaOH. Calc. S: 19.18; Found S: 19.05.

m,m'-Dithio-dibensoic acid was obtained from the corresponding mercaptan, which was synthesized by reduction of *m*-chlorosulphonylbensoic acid (4). The acid was purified by means of a continuous acetone extraction process as above. This acid is more soluble than the isomers. M.p. 247°–248°.

Anal. Subst., 48.83 mg; 4.66 ml of 0.0685*N* NaOH (bromothymol blue). Equ. wt. Calc. 153.1; Found 153.0. Subst., 41.44 mg; 4.08 ml of 0.0685*N* NaOH (phenolphthalein). Equ. wt. Calc. 153.1; Found 148.2.

Dimethyl *m,m'*-dithio-dibensoate was prepared as for the *o*-isomer from the *m*-acid and diazomethane. Recrystallizations from methanol, m.p. 44°–45°5.

Anal. Subst., 43.20 mg; 7.02 ml of 0.0733*N* NaOH. Calc. S: 19.18; Found S: 19.09.

p,p'-Dithio-dibensoic acid was obtained in a manner analogous to the *m*-isomer. The substance was purified according to both the methods described above. The *p*-acid has by far the lowest solubility of the isomers. M.p. 327°–330°.

Anal. Subst., 74.53 mg; 7.12 ml 0.0685*N* NaOH (bromothymol blue). Equ. wt. Calc. 153.1; Found 152.8. Subst., 43.24 mg; 4.19 ml of 0.0685*N* NaOH (phenolphthalein). Equ. wt. Calc. 153.1; Found 150.7.

Dimethyl-*p,p'*-dithio-dibensoate. Preparation as for the other isomers. The substance was repeatedly recrystallized from methanol. M.p. 124°–125°.

Anal. Subst., 32.74 mg; 5.34 ml of 0.0733*N* NaOH. Calc. S: 19.18; Found S: 19.16.

Di-*o*-nitrophenyl-disulphide was prepared from *o*-nitro-chlorobenzene and sodium disulphide (19) and purified by crystallizations from acetone. M.p. 194°–196°.

Summary

An investigation has been made of the ultraviolet and infrared absorption curves for the isomeric dithio-dibensoic acids (or their salts) and the corresponding dimethyl esters. The esters have been prepared from the acids with diazomethane.

The UV spectral behaviour is interpreted in terms of reasonable resonance forms. A special *o*-effect has been discussed and an explanation suggested.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor ARNE FREDGA for his great interest in the work and for kindly making available the *m,m'*-dithio-dibenzoic acid. The author is also indebted to Mr. GÖRAN BERGSON for valuable discussions.

The infrared absorption curves have been recorded by Dr. ANDREAS ROSENBERG with technical assistance from Mr. STIG BERGWALL.

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Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, October 1955.

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Uppsala 1956. Almqvist & Wiksells Boktryckeri AB

Spectrochemical studies on disulphides

III. An investigation of some 4-substituted 1,2-dithiolanes

By LENNART SCHOTTE

With 3 figures in the text

The first paper in this series on the spectrochemical behaviour of the disulphide linkage dealt with some linear and cyclic carboxylic substituted disulphides (1). The author intended to try to find a method of correlating the significant properties of different types of disulphides with a view to interpreting the behaviour of the special ring system 1,2-dithiolane. The influence of substituents like the carboxylic group in the α -positions (3,5-positions) to the S-S bond was then clearly illustrated and so the question arose whether substituents in the β -position (4-position) could have any influence on the spectral characteristics of this system.

As far as the author knows no carboxylic acid derivative of 1,2-dithiolane with the substituent in the 4-position is described in the literature. The disulphide related to β,β' -dimercapto-*iso*-butyric acid (2) is certainly mentioned but not isolated and characterized in any way. In fact, the molecular weight is questionable. It is hoped to be able to include the substance in this investigation series. There are, however, some other substances described in the literature, which allow a rather comprehensive study. Already in the years 1936-1938 BACKER and co-workers (e.g. 3, 4, 5) prepared a variety of compounds of spirane structure, some of which are derived from 1,2-dithiolane. 4,4-Dimethyl- and 4,4-bis-hydroxymethyl-1,2-dithiolane were also described. All these investigations have been performed with extreme experimental skill. The disulphides are in most cases obtained from primarily isolated trisulphides, the structure of which, however, will be discussed in the next paper of this series. Moreover, the molecular weight, a knowledge of which seems absolutely necessary in work with disulphides of this labile nature, has not been determined for any of the substances. Molecular weight determinations have now been made and confirmed completely the values assumed by BACKER. In connection with work on α,α' -dimercaptoacetone the present author described a semicarbazone of 1,2-dithiolanone-4 (6). This compound together with the others mentioned above has now been the subject of spectral analysis.

In Fig. 1 the ultraviolet absorption spectrum of 4,4-bis-hydroxymethyl-1,2-dithiolane (I) is shown. An absorption maximum is developed at $328\text{ m}\mu$ and a distinct inflexion in the $255\text{ m}\mu$ region. The absorption peak is thus found at nearly the same wavelength as that determined for the unsubstituted 1,2-dithiolane, $330\text{ m}\mu$ (7). The general picture of the absorption curves is moreover rather similar with the broad

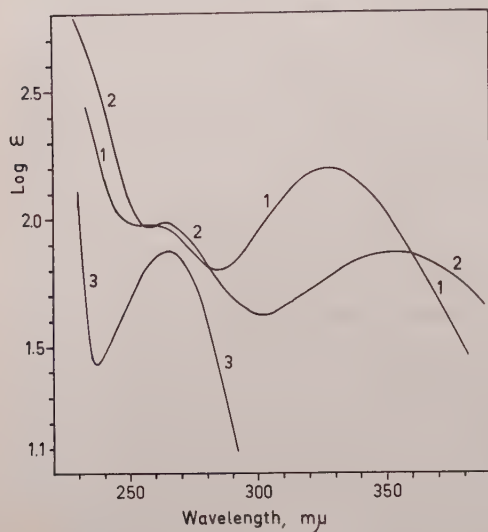


Fig. 1. UV absorption spectra of: (1) 4,4-bis-hydroxymethyl-1,2-dithiolane; (2) 2,6,7-trithia-(3,4)-spirooctane; (3) 2,6-dithia-(3,3)-spiroheptane. Solvent: alcohol.

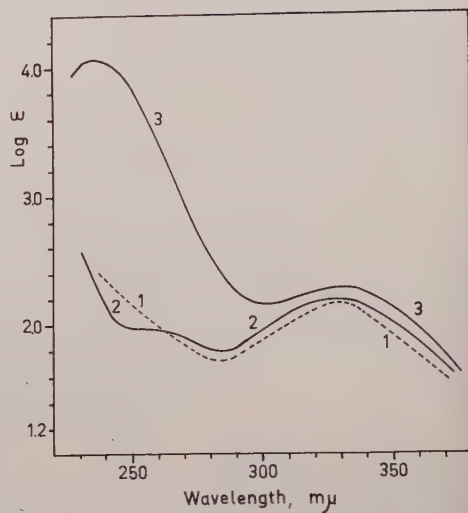
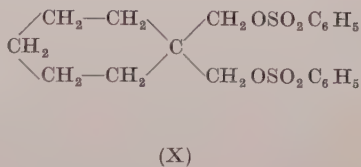
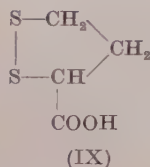
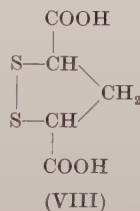
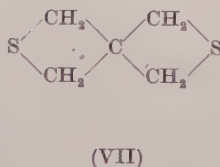
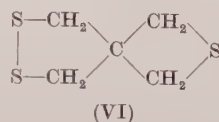
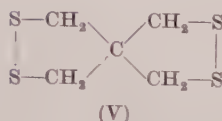
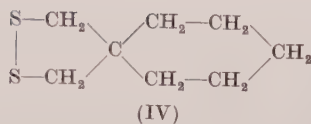
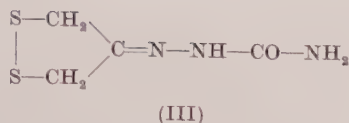
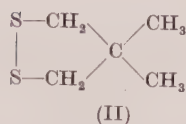
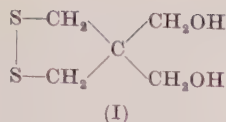


Fig. 2. UV absorption spectra in alcohol of: (1) 4,4-dimethyl-1,2-dithiolane (dashed curve); (2) 4,4-bis-hydroxymethyl-1,2-dithiolane; (3) 1,2-dithiolanone-4-semicarbazone.

maximum of low intensity in the 330 $m\mu$ region. However, differences are found at lower wavelengths, a fact surely due to the hydroxyl groups. This spectral detail will be further discussed later in connection with observations on the infrared absorption.

4,4-Dimethyl-1,2-dithiolane (II) is characterized by an UV absorption maximum at 331 $m\mu$. The curve is given in Fig. 2. It should be noted that the substance is rather unstable and easily polymerizes when quite pure. As it was possible for the present author to isolate the monomer in rather good purity for measurement, there is no special reason to doubt the appearance of the curve. In view of the results of BACKER, however, it seems justified to enter a reservation, as small amount of polymeric substances dissolved in the monomer might contribute essentially to the extinction determined. Fig. 2 gives also the absorption curve of 1,2-dithiolanone-4-semicarbazone (III), which further illustrates the characteristic selective absorption at 330 $m\mu$. In this wavelength region the spectrum resembles rather closely that of 4,4-bis-hydroxymethyl-1,2-dithiolane (I), here recorded again for comparison, but at lower wavelengths the difference is appreciable which of course is expected in view of the semicarbazone group (cf. 8). A high-intensity maximum is thus found here at 236 $m\mu$.

In all these three cases with substituents of different kinds the characteristic ultra-violet absorption of 1,2-dithiolane obviously remains unchanged. The specific absorption of disulphides is due to the excitation of the non-bonding p -electrons on the sulphur atoms and for linear disulphides usually found at 250 $m\mu$ (1, 9). For the cyclic five-membered disulphide a considerable bathochromic shift (to 330 $m\mu$) is found, which is explained by the fact that the nearly planar structure necessitates a changed azimuthal angle with a compression of the two p -electron orbitals as a consequence.



That means an increased repulsion between the two non-bonding *p*-electron pairs and thus excitation by less energy. When now carboxylic groups are inserted in the α -positions to the disulphide linkage the inductive effect of the substituent causes less electron density around the S-S bond than for the unsubstituted system, which is reflected in a hypsochromic shift. As the azimuthal angle is directly connected with the electronic conditions of the disulphide group, a change must consequently follow in the valency angles. Even if these changes are small the resultant effects may be pronounced because of the rigidity of the system. For the 4-substituted 1,2-dithiolanes, on the other hand, no mentionable deviation is found from the spectral behaviour of the unsubstituted disulphide. It thus follows from the foregoing that substitution in the 4-position does not imply any significant change in the electronic conditions for the S-S group in these cases. The effects of changed substitution at the 4-carbon atom are consequently not transmitted through the ring system.

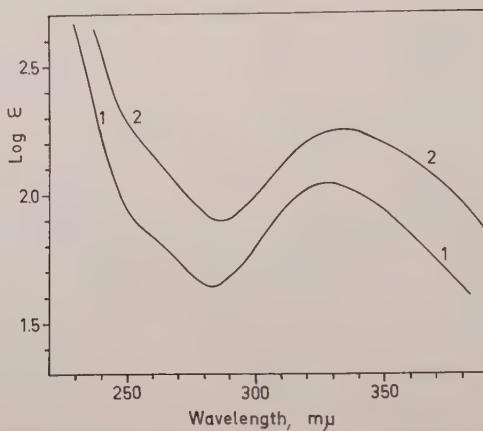


Fig. 3. UV absorption spectra in alcohol of: (1) 2,3-dithia-(4,5)-spirodecane; (2) 2,3,7,8-tetrathia-(4,4)-spirononane.

In fact this argument is mostly valid even if the substituent in the 4-position of 1,2-dithiolane is of cyclic structure. This is clearly demonstrated in Fig. 3, where the ultraviolet absorption spectra of two spiranes (IV and V) are given. 2,3-Dithia-(4,5)-spirodecane¹ (IV) shows the characteristic 1,2-dithiolane absorption at 329 $m\mu$. Somewhat lower intensity is, however, found for this bicyclic compound. 2,3,7,8-Tetrathia-(4,4)-spirononane (V) is very interesting from the spectral point of view, as it consists of two five-membered disulphide rings with one carbon atom (in the 4-position) in common. Although the absorption peak has exhibited a small shift towards longer wavelengths, the general appearance of this and the foregoing curve are closely similar. The maximum related to the S-S group is found here at 333 $m\mu$ and as could be expected the intensity is higher than for the compounds with only one 1,2-dithiolane ring. The small shift observed in specific absorption may indicate that a cyclic substitution involving strain in the molecule can influence the conditions around the disulphide linkage. Considerable change in the valency angles around the spiro-atom may thus be responsible for small changes in the characteristics of the ring system. This is well illustrated in Fig. 1, where the absorption curve for 2,6,7-trithia-(3,4)-spirooctane (VI) is also given. The effect is more pronounced here, as the maximum is developed at 354 $m\mu$. This, however, must be expected from the discussion above, as the spirane is composed of a five-membered cyclic disulphide and a four-membered ring sulphide with one atom in common. The UV absorption curve is also characterized by a slightly developed peak at 264 $m\mu$, which must be ascribed to the sulphide function in the thiacyclobutane ring. Unsubstituted thiacyclobutane has an ultraviolet peak at 272 $m\mu$ (7), whereas a related spirane, 2,6-dithia-(3,3)-spiroheptane (VII) exhibits a maximum at 265 $m\mu$. The latter curve is given in Fig. 1. In this wavelength region the agreement with 2,6,7-trithia-(3,4)-spirooctane (VI) in specific absorption is good. A comparison of the two spectra explains why the spirooctane derivative curve shows only a weakly developed peak at 264 $m\mu$ as the dithiolane system contributes strongly to the extinction in this region.

¹ Numbering according to Ch. A.

Thus the UV spectral analysis provides evidence for the close similarity between 1,2-dithiolane and most 4-substituted derivatives. An excitation of the sulphur *p*-electrons at even lower energy is found when the substitution implies considerable change in the valency angles.

Extending this spectrochemical investigation to include the infrared region it seems proper to call attention to the care which must be exercised in assigning absorption bands to carbon-sulphur or sulphur-sulphur vibrations (1, 10). The disulphide stretching frequency is generally assigned to $400\text{--}500\text{ cm}^{-1}$ ($25.0\text{--}20.0\text{ }\mu$) but for 1,2-dithiolane-3,5-dicarboxylic acid (VIII) the present author has found the unexpectedly high value 539 cm^{-1} ($18.57\text{ }\mu$) as probable (1). This indicates that the IR absorption of the S-S linkage in this special ring system is to be expected at higher frequencies than 500 cm^{-1} . Most of the 4-substituted 1,2-dithiolanes have been examined in the potassium bromide prism and partly even in that of sodium chloride. By careful analysis the spectra may be interpreted, but it should be pointed out that the following discussion is based upon conclusions drawn from a comparison of all the absorption curves obtained in this investigation. As the S-S peaks in general are regarded as ill-defined and difficult to characterize the uncertainty always arises whether the peak is detectable at all. Low intensities for the absorption peaks involve small changes in the dipole moment upon stretching of the group, which seems characteristic for disulphides in general. Due to the nature of this cyclic disulphide, however, there is reason to assume more pronounced dipole changes and thus distinct absorption. It has not been thought necessary to present any of the IR absorption curves as the frequency values found in Table 1 give sufficient data for interpretation.

The results of UV spectral analysis of 1,2-dithiolane-3,5-dicarboxylic acid (VIII) (1), 1,2-dithiolane-3-carboxylic acid (IX) (11) and the 4-substituted 1,2-dithiolanes investigated in this paper resp., involving bathochromic shifts of the specific absorption in the order given, may in this case suggest an analogous behaviour of the disulphide group towards infrared absorption. As the only available absorption peak in the possible wavelength region, at 539 cm^{-1} , is ascribed to the S-S group in 1,2-dithiolane-3,5-dicarboxylic acid (VIII) and the mono carboxylic acid derivative exhibits the corresponding frequency at 544 cm^{-1} , we could expect the 4-substituted ring compounds to show specific absorption at still higher frequencies. Thus 2,6,7-trithia-(3,4)-spirooctane (VI) probably has its S-S absorption band at 561 cm^{-1} ($17.82\text{ }\mu$), whereas the 2,3,7,8-tetrathia spirane (V) shows a peak at 570 cm^{-1} ($17.55\text{ }\mu$). 2,3-Dithia-(4,5)-spirodecane (IV), moreover, has the corresponding peak at 576 cm^{-1} ($17.37\text{ }\mu$). The relative order of magnitude in absorption frequency is certainly that expected but the differences within the 4-substituted series are, although small, remarkable. It is obvious that the frequency for the disulphide group stretching vibration varies to some extent even in closely related compounds.

In the previous discussion of the ultraviolet spectrum for 4,4-bis-hydroxymethyl-1,2-dithiolane (I) special attention was paid to the effect of the hydroxyl groups. An examination of the IR spectrum in the sodium chloride region reveals a long-wave shift of the characteristic OH-absorption of about 160 cm^{-1} to $3.07\text{ }\mu$. This displacement indicates some molecular association in the solid state through hydrogen bonding, which is of course possible between hydroxyl groups but also to a slight extent intermolecularly to a sulphur atom. Hydrogen bonding is not favoured in the case of sulphur compounds but would at least partly account for the fact that the S-S stretching vibration frequency is found at 530 cm^{-1} ($18.88\text{ }\mu$), which is a low

Table 1. The infrared absorption bands of some 4-substituted 1,2-dithiolanes. (The wavelength interval investigated differs somewhat for the substances.)

b = broad; vs = very strong; s = strong; m = medium; w = weak; sh = shoulder; e = emulsion peak.

4,4-Bis-hydroxy-methyl-1,2-dithiolane ¹			2,3-Dithia-(4,5)-spirodecane ²			2,6,7-Trithia-(3,4)-spirooctane ³			2,3,7,8-Tetrathia-(4,4)-spirononane ⁴			1,2-Dithiolanone-4-semicarbazone ⁵		
Wave-length in μ	Wave-number in cm^{-1}	Char-acter of the band	Wave-length in μ	Wave-number in cm^{-1}	Char-acter of the band	Wave-length in μ	Wave-number in cm^{-1}	Char-acter of the band	Wave-length in μ	Wave-number in cm^{-1}	Char-acter of the band	Wave-length in μ	Wave-number in cm^{-1}	Char-acter of the band
3.07	3257	vs	3.44	2907	vs	6.15	1626	w b	6.86	1458	e	12.07	829	w
3.42	2924	m	3.53	2833	vs	6.90	1449	m	7.10	1408	m	13.25	755	m
3.49	2865	m	3.77	2653	w	7.05	1418	s	7.26	1377	e	13.47	742	m
6.15	1626	w b	6.91	1447	vs	7.88	1269	s	7.88	1269	s	13.75	727	w
6.84	1462	m	7.09	1410	s	8.00	1250	s	7.98	1253	s	15.57	642	s
7.02	1425	s	7.47	1339	w	8.30	1205	s	8.2	1220	s b	16.65	601	vs
7.26	1377	m	7.60	1316	w	8.41	1189	m	9.00	1111	w	17.9	559	w b
7.66	1305	m	7.80	1282	s	8.55	1170	s	9.55	1047	m	19.9	503	s b
8.11	1233	m	8.00	1250	m	9.30	1075	m	9.97	1003	m	21.1	474	s b
8.39	1192	m	8.33	1200	s	9.57	1045	w	11.10	901	m	24.5	408	m b
9.19	1088	s	8.68	1152	w	9.75	1026	w	11.80	847	w			
9.65	1036	vs	8.97	1115	w	10.13	987	m	13.5	741	m b			
10.77	929	w	9.54	1048	w	10.88	919	m	13.89	720	e			
11.38	879	m	9.70	1031	w	11.22	891	w	14.42	693	s			
11.84	845	m	10.34	967	w	11.47	872	w	17.55	570	w			
13.65	733	m	10.57	946	m	11.80	847	w						
13.82	724	w b	10.73	932	m	12.40	806	w						
14.3	699	m	10.93	915	s	13.50	741	vs						
16.47	607	m	11.80	847	m	13.83	723	m						
18.88	530	w	12.15	823	w	14.60	685	w						
			12.35	810	w	15.05	664	w						
			13.60	735	m	17.82	561	m						
			13.88	720	m									
			14.9	671	sh									
			15.40	649	w									
			17.37	576	w									
			17.8	562	sh									

¹ Wavelength region 2-12 μ (NaCl prism); 11-24 μ (KBr prism). KBr phase.² Wavelength region 2-12 μ (NaCl prism); 11-23 μ (KBr prism). Liquid phase.³ Wavelength region 6-14 μ (NaCl prism), KBr phase; 11-23 μ (KBr prism). Nujol emulsion phase.⁴ Wavelength region 6-19 μ (NaCl prism); 11-23 μ (KBr prism). Nujol emulsion phase.

Table 2. Frequencies ascribed to the S-S stretching vibrations for some substituted 1,2-dithiolanes.

Substance	Wavelength, μ	Wavenumber, cm^{-1}	Reference
1,2-dithiolane-3,5-dicarboxylic acid	18.57	539	(1)
1,2-dithiolane-3-carboxylic acid . . .	18.37	544	(11)
4,4-Bis-hydroxymethyl-1,2-dithio- lane	18.88	530	This paper
2,3-Dithia-(4,5)-spirodecane	17.37	576	" "
2,6,7-Trithia-(3,4)-spirooctane . . .	17.82	561	" "
2,3,7,8-Tetrathia-(4,4)-spirononane	17.55	570	" "

value as compared with those mentioned above. Evidence has recently been presented for the existence of hydrogen bonding from a hydroxyl group to a sulphur atom in a 1,4-dithiane derivative (12).

The frequencies thus ascribed to the S-S stretching vibrations are collected in Table 2. It should finally be emphasized that the carbon-sulphur frequency is found here in the region around 14μ , often as double peaks. As the assignment involves uncertainty because of structural vibrations in the vicinity no further discussion is given. The complicated character of the infrared absorption curve in the region $11\text{--}25 \mu$ for 1,2-dithiolanone-4-semicarbazone (III) excludes any interpretation.

Experimental

A Beckman model DU quartz spectrophotometer was used for the *ultraviolet absorption determinations*. Alcohol was used as a solvent and the curves were recorded two or three times.

For the *infrared absorption measurements* a Perkin Elmer model 21 spectrophotometer was used. The substances were investigated as solid pressed potassium bromide plates (13), as Nujol emulsions or in liquid phase. For the sodium chloride region the calibration is correct to $\pm 0.02 \mu$ and for longer wavelengths to $\pm 0.04 \mu$.

The *molecular weight determinations* were performed according to the ebullioscopic method with acetone or benzene as solvents. The apparatus used was a modified molecular weight apparatus for semimicro analyses. (A product of Emil Greiner & Co. (14) was improved some years ago by Docent BRÄNDSTRÖM, Mr. ÖHBERG and the present author in collaboration and has since then been successfully used at this institute.) A calibration is done with a known substance and the observed elevations of the boiling point are plotted against the amounts of sample. In these determinations amounts up to $0.1\text{--}0.2 \text{ g}$ (total) were used.

4,4-Bis-hydroxymethyl-1,2-dithiolane was prepared according to BACKER and TAMMSMA (3) (diiodopentaerythritol was used instead of the analogous bromo compound) and recrystallized twice from benzene. M.p. 129° . Mol. wt. (benzene) Calc. 166.3; Found 160.

4,4-Dimethyl-1,2-dithiolane was obtained as described by BACKER and TAMMSMA (3). It was, however, found advisable to extend the treatment of the corresponding trisul-

phide with copper to 30 minutes. Careful fractionation yielded a substance, which polymerized easily. B.p. 82° – 83° /12 mm. Mol. wt. (benzene) Calc. 134.3; Found 131.

1,2-Dithiolanone-4-semicarbazone. The preparation is described in (6), where further data are given. M.p. 223° – 224° .

2,6,7-Trithia-(3,4)-spirooctane. Preparation according to BACKER and EVENHUIS (4). The reaction time with copper was, however, extended to 60 minutes or the primary procedure repeated. The sulphide was recrystallized several times from mixtures of ether and light petroleum (b.p. 40° – 60°). M.p. 53° – 54° . Mol. wt. (acetone) Calc. 164.2; Found 169.

2,3-Dithia-(4,5)-spirodecane. Preparation according to BACKER and TAMSMA (3). Fractional distillation *in vacuo* yielded a substance of good purity, b.p. 151° /19 mm. Mol. wt. (acetone) Calc. 174.3; Found 170.

The dibromide used for this synthesis, 1,1-bis-bromomethyl-cyclohexane, was obtained from the corresponding hydroxy derivative (15) in reaction with benzene-sulphonyl chloride and subsequent attack on the ester formed by sodium bromide in diethylene glycol as solvent. This method is superior to that described by BACKER and WINTER (15). The method employed is analogous to that for the synthesis of pentaerythrityl tetrabromide (16). It seems therefore unnecessary to give the experimental details. As an intermediate product *1,1-bis-hydroxymethyl-cyclohexane di-benzenesulphonate* (X) was isolated. Recrystallizations twice from alcohol. M.p. 101° – 102° .

Anal. Subst., 22.48 mg; 2.90 ml of 0.0733N NaOH. $C_{20}H_{24}O_6S_2$ (424.5). Calc. S 15.10; Found S 15.16.

2,3,7,8-Tetrathia-(4,4)-spirononane was prepared according to BACKER and EVENHUIS (5) and repeatedly recrystallized from mixtures of alcohol and benzene. M.p. 79° – 80° . Mol. wt. (acetone) Calc. 196.3; Found 199.

2,6-Dithia-(3,3)-spiroheptane was prepared from pentaerythrityl tetrabromide and K_2S in alcoholic solution (17). Upon distillation a colourless substance, b.p. 103° – 104° /12 mm was obtained, which soon crystallized to form large plates. M.p. 31° – 32° .

Summary

The ultraviolet and infrared absorption curves of some 4-substituted 1,2-dithiolanes have been investigated.

The close similarity between the UV absorption behaviour of 1,2-dithiolane and the 4-substituted derivatives has been discussed.

1,1-Bis-hydroxymethyl-cyclohexane di-benzenesulphonate has been prepared.

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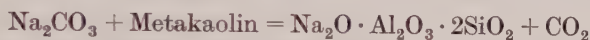


Über die Synthese einiger Skarnminerale aus den pulverförmigen Komponenten

Von ROBERT JAGITSCH

Mit 4 Figuren im Text

Seit einigen Jahren wurden am Institut für Silikatforschung in Göteborg auch solche Reaktionen zwischen festen Stoffen untersucht, welche insbesondere geologisches Interesse besitzen. Dabei wurde vorerst die Bildung von Alkali-Aluminiumsilikaten, wie zum Beispiel die von Nephelin entsprechend der Umsetzung



und die Bildung von Natrium-Kalium-Feldspatmischkristallen durch Umsetzung von Albit und Orthoklas in trockenen Pulvergemischen studiert (1, 2). Die Diffusionsgeschwindigkeiten in diesen Systemen sind verhältnismässig gross und eine quantitative Bestimmung derselben leicht möglich. So wurde beispielsweise die Diffusionskonstante bei der Alkalidiffusion unter Feldspatmischkristallbildung bei 1000°C zu $5 \cdot 10^{-10} \text{cm}^2 \text{sec}^{-1}$ gefunden.

In Systemen mit wandernden Erdalkalien liegen beachtlich geringere Diffusionsgeschwindigkeiten vor als bei solchen mit Alkalien. Mit noch langsameren Materialverschiebungen muss in Oxydsystemen mit mehrwertigen Reaktionskomponenten gerechnet werden. Ein Beispiel hierfür sind die Prozesse bei der Erhitzung von Kaolin, wobei die Bildung von Mullit aus Al_2O_3 und SiO_2 erst bei Temperaturen nahe 900° merkbar wird und dies trotz der denkbar besten, nämlich molekularen Mischung der Komponenten — einer idealen Mischung der Komponenten, wie sie durch mechanisches Vermengen nie und durch Zusammenfällungsoperationen der Reaktionspartner nur unvollständig erzielt werden kann.

Die im folgenden beschriebenen Bildungsreaktionen der skarnegeologisch interessanten Granate und des Diopsids wurden nur in qualitativer Hinsicht untersucht. Die Umsetzungen wurden mit Material geringster Korngrösse ausgeführt um die Bildung dieser Stoffe unter den günstigsten Bedingungen studieren zu können. Es wurden daher gefälltes Kalziumkarbonat (Merck p. a.), gefällte Kieselsäure (Baker p. a.), gefälltes Aluminiumhydroxyd (Baker) verwendet. Als EisenIII-oxyd zur Andraditbildung kamen eine ganze Reihe von Präparaten zur Anwendung, nämlich selbst gefälltes Hydroxyd, aus Eisensulfat bzw. Eisenoxalat durch Zersetzung an der Luft bei 650 respektiv 1050°C gewonnen und schliesslich Fe_2O_3 , Merck.

Eine Berechnung von Reaktionsgeschwindigkeiten ist in diesen Untersuchungen praktisch nicht möglich, nachdem die verwendeten Korngrössen zu gering sind und dazu in weiten Grenzen variieren. Ziel der Untersuchungen war vorläufig die qualitative Feststellung des Auftretens der gesuchten Phasen unter Variation von Tem-

peratur, Feuchtigkeit und Druck, komplettiert durch Bestimmungen der Beständigkeit dieser synthetisch gebildeten Phasen beim Wiederhitzen. Die Umsetzungen wurden in mittels Handpresse zu Pastillen gepressten Pulvergemischen durchgeführt.

1. Diopsidbildung

Bei Erhitzung trockener Pulvergemische aus CaCO_3 , MgO und SiO_2 an der Luft kann mittels Röntgenuntersuchung die Bildung von Diopsid bei Erhitzungszeiten von 24 Stunden erst bei 1200°C nachgewiesen werden. Die bei Erhitzung auf 1000° und 1100°C allein beobachtbaren Interferenzen von CaO und MgO sind bei 1200° verschwunden und statt diesen — neben Cristobalit — die von Diopsid deutlich sichtbar. Bei 1300° tritt Diopsid — immer noch durch Umsetzung im festen Zustand gebildet — allein im Röntgenogramm auf; bei 1325° ebenfalls, aber gröber kristallisiert, entsprechend der Bildung in der Schmelze.

Beim Überleiten von Wasserdampf von circa einer Atmosphäre kann die Bildung von Diopsid bereits bei 1000° festgestellt werden. CaO - und MgO -Linien, welche bei der Erhitzung des Gemisches im Wasserdampf auf 900° und beim Erhitzen im trockenen Luftstrom selbst bei 1100° noch allein nachweisbar sind, sind völlig verschwunden. Die Bildung von Diopsid ist jedoch auch im Wasserdampfstrom bei 1100° nicht allein vorherrschend, sondern es erfolgt in Konkurrenz dazu eine Kristallisation der gefällten Kieselsäure zu Cristobalit. Erst bei 1200° wird in feuchter Atmosphäre, entsprechend der stöchiometrischen Zusammensetzung des Pulvergemisches ausschliesslich Diopsid gebildet.

Der günstige Einfluss des Wasserdampfes tritt am deutlichsten bei der Erhitzung des Pulvergemisches im Autoklaven hervor: bei einem Wasserdampfdruck von 500 Atmosphären wurde vollständige Umsetzung des Pulvergemisches zu Diopsid bereits nach 24-stündiger Erhitzung auf nur 520°C festgestellt. Bei 400° aber und einem Wasserdampfdruck von etwa 300 Atmosphären liegen auch nach 65 Stunden noch unverändert die Interferenzen der Ausgangsstoffe vor (CaCO_3 und MgO ; die gefällte Kieselsäure ist amorph).

In der folgenden Tabelle 1 sind die Bildungsverhältnisse zusammengestellt. Die Versuchsdauer war in sämtlichen Versuchen, mit Ausnahme der Erhitzung unter hohen Wasserdampfdrucken, 24 Stunden; im Falle der Erhitzung im Autoklaven wurde die Versuchsdauer auf 65 Stunden ausgestreckt.

2. Granatbildung

Die Bildung von Granat ist bereits am System $\text{CaO-Al}_2\text{O}_3\text{-SiO}_2$ von YODER (3) untersucht worden, welcher dabei zu der Auffassung gelangte, dass Grossular vermutlich bei Atmosphärendruck stabil sei, sich auch prinzipiell bei gewöhnlichem Druck aus den Oxyden bilden könne und erhöhter Druck nur die Bildung beschleunige. Ferner: dass die meisten als Grossulare beschriebenen Minerale in Wahrheit Hydrogrossulare seien, da in Anwesenheit von Wasserdampf nur diese gebildet werden könnten.

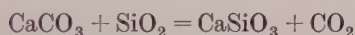
Für das vorliegende Problem der Skarmineralbildung war vor allem die Synthese des Andradits, $3\text{CaO}\cdot\text{Fe}_2\text{O}_3\cdot 3\text{SiO}_2$, von Interesse. Anschliessend wurde ferner die Bildung von Uvarovit, $3\text{CaO}\cdot\text{Cr}_2\text{O}_3\cdot 3\text{SiO}_2$, untersucht.

Tabelle 1. Bildung von Diopsid unter verschiedenen Bedingungen.

Spezielle Versuchsbedingungen	Temperatur °C	Röntgenresultat
Erhitzung an der Luft; Wasserdampfdruck etwa 12 mm; Gesamtdruck 1 Atmosphäre	1000	CaO, MgO
	1100	CaO, MgO
	1200	Diopsid, Cristobalit
	1300	Diopsid
	1325	Diopsid (Schmelze)
Erhitzung im Wasserdampfstrom; Gesamtdruck 1 Atmosphäre, Wasserdampfdruck etwa 0,7 Atmosphären	900	CaO, MgO
	1000	Diopsid, Cristobalit
	1100	Diopsid, Cristobalit
	1200	Diopsid
Gesamtdruck und Wasserdampfdruck etwa 300 Atmosphären	400	CaCO ₃ , MgO
Gesamtdruck und Wasserdampfdruck etwa 500 Atmosphären	520	Diopsid

a, Untersuchungen am System CaO-Fe₂O₃-SiO₂

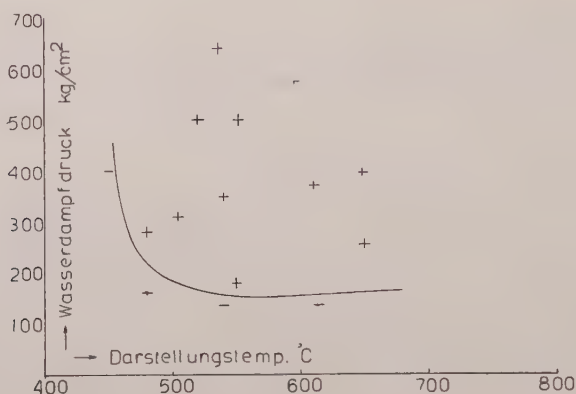
Erhitzungen von zu Pastillen gepressten Pulvergemischen aus CaCO₃, Fe₂O₃·xH₂O, und SiO₂·xH₂O bei gewöhnlichem Druck führte weder in trockener Atmosphäre noch in Wasserdampf bei Temperaturen bis 1280°C und Versuchszeiten bis zu 720 Stunden zur Andraditbildung. Eine gewisse reaktionsbefördernde Wirkung von Wasserdampf zeigte sich doch auch bei diesen Versuchen darin, dass die Bildung von Wollastonit nach



unter den vorliegenden Bedingungen im Wasserdampf bereits bei 1100°C mit der gleichen Intensität erfolgt, wie bei trockener Erhitzung erst bei um etwa hundert Grad höherer Temperatur.

Im Autoklaven wurden hingegen bei Wasserdampfdrucken von mehr als etwa 150 Atmosphären in einem breiten Temperaturintervall Andraditbildung festgestellt. Die Versuchszeiten lagen in der Regel zwischen 60 und 150 Stunden; nur ausnahmsweise wurde eine längere Versuchsdauer (500 und 525 Stunden) gewählt. Eine Versuchsdauer von nur 20 Stunden erwies sich bei sonst günstigen Bedingungen (Temperatur, Wasserdampfdruck) als unzureichend.

Die Versuchsergebnisse dieser Erhitzungen unter Variation der Versuchstemperatur und des Wasserdampfdruckes werden in der folgenden Fig. 1 zusammengefasst, wobei das röntgenographisch festgestellte Vorliegen von Andradit mit einem Pluszeichen, ein Nichtantreffen von Andradit mit einem Minuszeichen eingezeichnet wird. Wie die Figur zeigt, wird dabei ein Feld der innerhalb der Versuchszeiten erfolgenden Andraditbildung erhalten, welches oberhalb etwa 150 Atmosphären Wasserdampfdruck und oberhalb etwa 480°C liegt. Eine Verlängerung der Versuchszeiten auf 525 Stunden hingegen ergab deutlich Andraditbildung bereits bei nur 110 Atmo-



sphären Wasserdampfdruck. Die Erhitzungstemperatur war 660°C. Das Feld der Andraditbildung wird also durch Verlängerung der Erhitzungszeiten erweitert.

Wie oben angeführt, wurden in den Reaktionsgemischen verschiedene Eisenoxydpräparate verwendet. Andraditbildung wurde unter den eben besprochenen Reaktionsverhältnissen festgestellt in Gemischen mit gefällttem Eisenhydroxyd und bei Eisenoxyden, welche durch thermische Zersetzung von Oxalat oder Sulfat gewonnen waren, hingegen *nicht* in Gemischen mit Fe_2O_3 Merck (Urtitersubstanz). Der Grund dafür konnte bisher nicht ermittelt werden.

Der im Autoklaven bei hohen Wasserdampfdrucken synthetisierte Andradit enthält geringe Mengen Wasser, welche erst bei Erhitzen auf Temperaturen oberhalb etwa 700°C rascher abgegeben wird. Selbst bei 48stündiger Erhitzung auf 650°C wird nur etwa ein Drittel dieses Wassergehaltes abgegeben—vermutlich auch das grösstenteils adsorptiver Art und daher nicht so fest gebundene wie das erst bei höherer Temperatur abgespaltene Wasser. Die folgende Tabelle 2 gibt Aufschluss über diese Entwässerungsversuche synthetischer Andraditpräparate unter verschiedenen Bedingungen der Temperatur und Erhitzungsdauer.

Gleichzeitig mit dieser Wasserabgabe bei höheren Temperaturen beobachtet man eine deutliche Linienverschiebung im Röntgenogramm im Sinne einer Kontraktion

Tabelle 2. Wasserabgabe und Gitterschrumpfung von synth. Andradit beim Erhitzen.

Erhitzungs- temperatur, °C	Erhitzungsdauer, h	Wasser abgegeben, Gew.-%	Kantenlänge der Elementarzelle, Å
nicht erhitzt	—	0,0	12,09
430	4	0,4	nicht bestimmt
500	1	0,9	nicht bestimmt
650	48	0,7	12,09
1000	2	1,9	12,05
1000	22	2,5	nicht bestimmt
1080	2	2,5	12,03
1220	1	2,5	12,02
1300	1	2,5	zerfallen in Fe_2O_3 und CaSiO_3

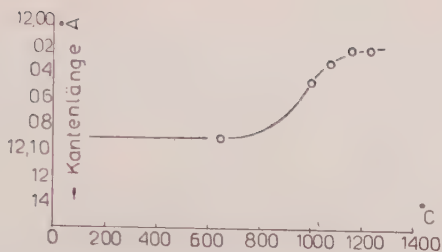


Fig 2b.

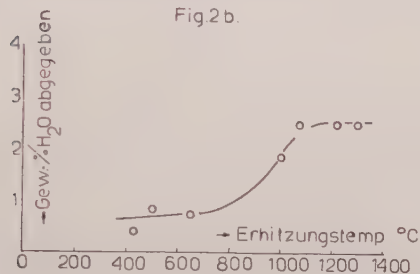


Fig 2a.

der Elementarzelle. Die Abnahme der Kantenlänge beim Erhitzen von synthetischem Andradit an der Luft bei gewöhnlichem Druck ist ebenfalls in Tabelle 2 aufgenommen und graphisch in Figur 2 wiedergegeben. In dieser wurde gegen die Temperatur als Abzisse auf der Ordinate in 2a das abgegebene Wasser (in Gewichtsprozenten) und in 2b die jeweilige Kantenlänge der Elementarzelle aufgetragen.

b. Untersuchungen am System $\text{CaO}-\text{Cr}_2\text{O}_3-\text{SiO}_2$

In analoger Weise wie die Andraditbildung wurde durch Erhitzen von $\text{CaCO}_3-\text{Cr}_2\text{O}_3-\text{SiO}_2$ -Presslingen im Autoklaven die Bildung von Uvarovit unter verschiedenen Bedingungen der Temperatur und des Wasserdampfdruckes untersucht. Die dabei

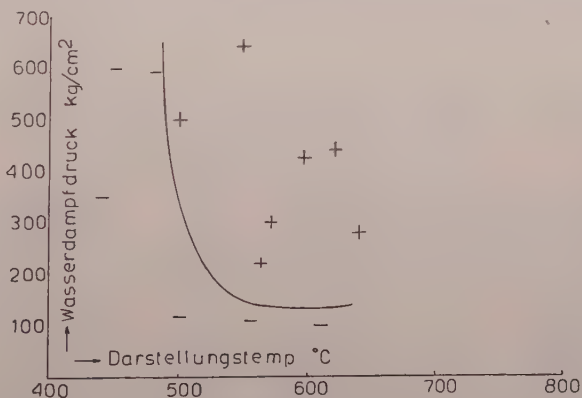


Fig 3

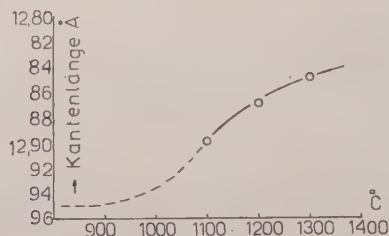


Fig. 4b

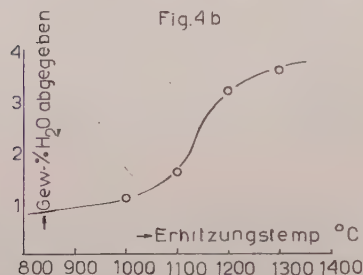


Fig. 4a

röntgenographisch festgestellten Bildungsverhältnisse (bei Versuchszeiten zwischen 60 und 140 Stunden) gehen aus der Fig. 3 hervor.

Eine Verlängerung der Erhitzungsdauer auf 525 Stunden ergab bei 110 Atmosphären Wasserdampfdruck und einer Temperatur von 660° im Gegensatz zum System $\text{CaO-Fe}_2\text{O}_3\text{-SiO}_2$ keine Granatbildung. Dass das „Bildungs“-Feld für Uvarovit ebenso wie das des Andradites keineswegs ein Feld im Sinne eines thermodynamischen Gleichgewichts darstellt, geht z. B. daraus hervor, dass eine Erhitzung von Uvarovit nahe *ausserhalb* dieses Feldes (400°C, 245 Atmosphären Wasserdampfdruck, 117 Stunden) keineswegs zu einer Zerstörung desselben führt. Bei sehr hohen Temperaturen hingegen wird Uvarovit zersetzt wie in der Folge gezeigt wird.

Ebenso wie der synthetische Andradit ist auch der synthetisch unter hohen Wasserdampfdrucken hergestellte Uvarovit wasserhaltig. Die Abgabe dieses wieder recht fest gebundenen Wassers beim Erhitzen unter gewöhnlichem Druck an der Luft

Tabelle 3. Wasserabgabe und Gitterschrumpfung beim Erhitzen von synthetischem Uvarovit.

Erhitzungstemperatur, °C	Wasserabgabe, Gew.-%	Kantenlänge der Elementarzelle, Å
nicht erhitzt	0,0	11,95
1000	1,1	nicht bestimmt
1100	1,6	11,90
1200	3,2	11,87
1300	3,6	11,85
1400	nicht bestimmt	zerfallen in Cr_2O_3 und CaSiO_3

sowie die damit verbundene Schrumpfung der Elementarzelle ist in der Tabelle 3 wiedergegeben. Aus der graphischen Darstellung in Fig. 4a und b geht noch deutlicher hervor, dass die Gitterschrumpfung an die Wasserabgabe bei hoher Temperatur geknüpft ist. Die Erhitzungsdauer war durchwegs 2 Stunden.

3. Zusammenfassung

Die Synthese skarngeologisch interessanter Mineralien in Abhängigkeit von Bildungstemperatur und -dauer sowie des Einflusses des Wasserdampfdruckes wurde untersucht.

Diopsid, Andradit und Uvarovit wurden dargestellt und auf deren Beständigkeit untersucht.

Die synthetischen Granate enthalten stark gebundenes Wasser, welches erst bei hoher Temperatur, unter Schrumpfung des Gitters, abgegeben wird.

Für Anregung und Rat möchte ich auch an dieser Stelle Överdirektör Professor NILS MAGNUS-SON danken. Ferner bin ich dem Vorstand des Silikatforschungsinstitutes, Herrn Professor J. A. HEDVALL, welcher für die Durchführung dieser Untersuchungen materielle Unterstützung von Statens naturvetenskapliga Forskningsfond erhalten hat, zu Dank verpflichtet. Mein Dank gilt endlich den Ingenieuren VURAL BAKANAY und MANNE LÖVENBY für ihre Hilfe bei der Durchführung der Versuche und Svenska Oljeslageri A.B., Mölndal, für die Überlassung eines Autoklaven.

Institut für Silikatforschung, Göteborg.

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Tryckt den 1 mars 1956

Uppsala 1956. Almqvist & Wiksells Boktryckeri AB



Ergebnisse an Brenztraubensäure

Von HANS VON EULER und HANS HASSELQUIST

I. Oxydative Decarboxylierung der Brenztraubensäure durch Hydroperoxyd

Während die Brenztraubensäure in der Hefe durch die Carboxylase (mit Aneurin-pyrophosphat als Coenzym) in Acetaldehyd und CO_2 zerlegt wird, verläuft die Brenztraubensäure-Spaltung in der tierischen Zelle¹ bekanntlich durch die Decarboxylase (mit Lipothiamid-pyrophosphat als Coenzym) oxydativ unter Bildung „aktivierter“ Essigsäure und CO_2 .

Dieser Vorgang entspricht im wesentlichen der nicht-enzymatischen oxydativen Decarboxylierung durch Hydroperoxyd.

Versuchs-Serie I

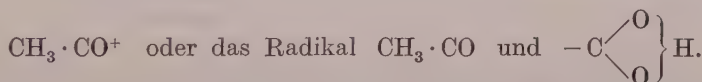
Aus 1 Mol Brenztraubensäure + 1 Mol Hydroperoxyd bei 20° in 18 Stunden in wässriger Lösung entstehen

0,9 Mol CO_2 und 1 Mol Essigsäure,

nahezu übereinstimmend mit der Gleichung



Bei der Decarboxylierung der Brenztraubensäure kommt also als Zwischenform das Radikal $\text{CH}_3 \cdot \text{CO}$ oder das Kation $\text{CH}_3 \cdot \text{CO}^+$ in Betracht. Das Ion $\text{CH}_3 \cdot \text{CO}^+$ wurde als das zum Essigester-Gleichgewicht führende Zwischenprodukt vor langer Zeit vom einen von uns (H. v. E.) in Rechnung gezogen². Es treten also bei der oxydativen Decarboxylierung der Brenztraubensäure auf:



Im System Ascorbinsäure + Hydroperoxyd + Äthanol + Ferrichlorid, in dem als Oxydationsprodukte Acetaldehyd und Essigsäure auftreten, zeigt sich deutlich die Rolle der Ascorbinsäure als Oxydationskatalysator³. Bei diesen Versuchen wie

¹ Brenztraubensäure wurde im Muskel zuerst von CASE und COOK (Biochem. J. 36, 1316; 1931) nachgewiesen.

² EULER, z. physikal. Chem. 36, 405 u. 641 (1901).

³ EULER u. H. HASSELQUIST, Z. physiol. Chem. 303, im Druck (1956).

auch bei denen über den Einfluss von Ascorbinsäure + Hydroperoxyd auf die Viscosität von Pektinlösungen¹ wird man die Bildung eines Komplexes Ascorbinsäure-Hydroperoxyd bzw. eines Dehydro-ascorbinsäure-peroxydes annehmen.

Bei der oxydativen Decarboxylierung der aus Dehydro-ascorbinsäure durch Ringöffnung entstehenden Diketogulonsäure bildet sich weder in wässrig-alkoholischer noch in rein wässriger Lösung Acetaldehyd; man findet ausser CO₂ noch Oxalsäure und Monocarbonsäuren (Essigsäure, Ameisensäure); sowohl in diesem Fall wie bei der Oxydativen Decarboxylierung der Brenztraubensäure kann man die Aktivierung der Hydroperoxyds auf einen elektrostatischen Induktionseffekt zurückführen.

Bei der oxydativen Decarboxylierung der Brenztraubensäure durch Bakterien fand LIPMANN Acetylphosphat.

Was die Wirkung des Hydroperoxyds auf Äthanol betrifft, so ergab ein Versuch, dass sich bei Gegenwart von Fe⁺⁺⁺ aus 16 Molen Äthanol + 1 Mol Hydroperoxyd bei 20° in 18 Stunden 0,38 Mole Essigsäure bilden.

In Anwesenheit von Fe⁺⁺⁺ wird auch bei geringerer Äthanol-Konzentration durch Hydroperoxyd (in saurer Lösung) ein kleiner Teil des Äthanol zu Essigsäure oxydiert (kein Acetaldehyd).

2 Mole Äthanol + 1 Mol H₂O₂ + Fe⁺⁺⁺ geben 0,08 Mol Essigsäure.

In wässriger Lösung ohne Oxydationsmittel ist Brenztraubensäure innerhalb eines weiten pH-Gebietes unter 50° bekanntlich recht stabil. Als Katalysatoren der Decarboxylierung können bekanntlich Amine fungieren, besonders aromatische, wobei Schiffsche Basen entstehen.²

An der Dehydrierung, welche der enzymatischen Decarboxylierung im Tierkörper vorausgehen muss, sind vermutlich mehrere Cozymase-bedingte Dehydrasen beteiligt. Wesentlich ist die Wasserstoff-Übertragung durch die Dihydro-Cozymase, CoH₂.

Im Anschluss an Versuchsserie I liessen wir Hydroperoxyd auf Brenztraubensäure in 96 %iger Äthanol-lösung einwirken, um zu untersuchen, ob Brenztraubensäure die Oxydation von Äthanol, die *direkt nicht* eintritt, katalysiert.

Versuchs-Serie II

Brenztraubensäure wurde in Gegenwart von Äthanol und Fe⁺⁺⁺ durch Hydroperoxyd in folgender Reaktionslösung decarboxyliert:

Brenztraubensäure (monomer)		44 mg	0,500 mMole	} 20 ml
Hydroperoxyd 30 %ig		0,11 ml	1,00 mMol	
Äthanol		0,2 ml	2,00 mMole	
Fe ⁺⁺⁺			0,013 mMole	

Aus der Reaktionslösung entwickelten sich 0,250 mMole CO₂. Im Destillat der Reaktionslösung fanden sich 0,352 mMole Essigsäure. (Ameisensäure konnte nicht nachgewiesen werden.)

1 Mol Brenztraubensäure + 2 Mole Hydroperoxyd + 4 Mole Äthanol gaben also bei 20° in 18 St. 0,83 Mole CO₂ und 0,70 Mole Essigsäure.

¹ M. L. STEIN u. EULER, Z. makromolekulare Chemie 15, 60 (1955). — EULER u. H. HASSELQUIST, Dieses Arkiv, 8, nr 37 (1955). — Makromolekulare Chemie 17 (1956).

² EULER, H. HASSELQUIST u. E. ERIKSSON, Liebigs Ann. Chem. 578, 188 (1952). — Siehe auch J. P. GREENSTEIN u. C. I. CARTER, J. Biol. Chem. 175, 523 (1948).

Es wurde demnach in Betracht gezogen, dass Äthanol durch Brenztraubensäure bei der Decarboxylierung (katalytisch durch das Radikal $\text{CH}_3\cdot\text{CO}$) zu Essigsäure oxydiert wird.

Über sek. Oxydation von Alkohol siehe D. KEILIN u. E. F. HARTREE.¹

Versuchs-Serie IIb

Die folgende Versuchs-Serie IIb wurde *ohne* Zusatz von FeCl_3 angestellt, und in den Destillaten die entstandenen Mengen Säure verglichen, die sich aus der Brenztraubensäure in Gegenwart von Äthanol (Vers. 3–6) und Abwesenheit von Äthanol (Vers. 7–10) bei 20° und 16 St. gebildet haben.

Reaktionslösung:

Brenztraubensäure (frisch), wässr. Lös. 18,6 mg/ml

H_2O_2 30 %ig

Äthanol 96 %ig

Versuche Nr.	CH_3COCOCH ml	Wasser ml	Äthanol ml	H_2O_2 ml	Gebildete Säure- Äquivalente
1	2,0	1,0	2,0	—	4,55
2	2,0	3,0	—	—	4,55
3	2,0	0,9	2,0	0,1	4,60
4	2,0	0,8	2,0	0,2	4,60
5	2,0	0,6	2,0	0,4	4,70
6	2,0	0,2	2,0	0,8	4,80
7	2,0	2,9	—	0,1	4,55
8	2,0	2,8	—	0,2	4,55
9	2,0	2,6	—	0,4	4,55
10	2,0	2,2	—	0,8	4,60

Die Versuchs-Serie IIb zeigt, dass Äthanol unter dem katalytischen Einfluss von $\text{CH}_3\cdot\text{CO}$ nur zu einem *ganz kleinen Teil*, in Spalte 6 Differenz 4,75–4,55, zu Essigsäure oxydiert wird.

Versuchsserie III

An einem anderen Substrat, nämlich an Glucose², wurde untersucht, ob die Brenztraubensäure (bzw. ihr Spaltprodukt $\text{CH}_3\cdot\text{CO}$) bei der Oxydation durch Hydroperoxyd als Katalysator fungieren kann. Dies ist unter den gewählten Versuchsbedingungen auch bei grossem Überschuss an Glucose *nicht* der Fall: Es wurde in Versuchs-Serie III keine grössere Menge Essigsäure gebildet, als es der Menge der angewandten Brenztraubensäure entspricht. In Versuchs-Serie III enthielt die Reaktionslösung (20 ml) auf 0,50 mMole Brenztraubensäure 2 mMole Hydroperoxyd und rund 1 g Glucose.

Eine oxydative Decarboxylierung, wie im System Brenztraubensäure, Hydroperoxyd, Äthanol und Fe^{+++} (Versuchs-Serie II), tritt nur in viel geringerem Grad und langsamer ein, wenn Hydroperoxyd durch Perjodsäure oder durch Chromsäure oder andere Oxydationsmittel ersetzt wird. Eine Dehydrierung der Brenztraubensäure wird durch gewisse Farbstoffe beeinflusst.

¹ D. KEILIN u. E. F. HARTREE, Biochem. J. 60, 310 (1955).

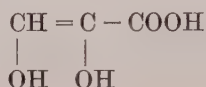
² Glucose wurde zu diesem Versuch gewählt in der Erwägung, dass durch Vermittlung der Brenztraubensäure eine Spaltung der Glucose eintreten könnte ähnlich der durch Bleitetraacetat (GRIEGEE) oder durch Perjodsäure (P. FLEURY u. J. LANGE) erreichten. Siehe hierzu P. KARRER u. K. PFAHLER, Helv. Chim. Acta 17, 363 (1934).

Offenbar handelt es sich dabei nicht um eine unspezifische Oxydation, sondern um eine Dehydrierung unter Vermittlung eines Brenztraubensäure-Hydroperoxyd-Komplexes (ev. einer Brenztrauben-persäure).

Ob der oxydative Verlauf der Brenztraubensäure-Decarboxylierung bei der zum Citronensäure-Cyklus führenden „Aktivierung“ der Essigsäure¹ im Tierkörper durch spezifische Dehydrasen oder durch die freie Cozymase selbst oder durch ein anderes enzymatisches Pyruvat-Oxydase-System vermittelt wird, steht noch nicht fest. Durch letzteres System kann Aneurin mit der Decarboxylierung der Brenztraubensäure im Sinne von R. A. PETERS² verknüpft sein.

Oxydation des Triose-Reduktions durch Hydroperoxyd

Triose-Reduktion liefert bei der Oxydation mit Jod Mesoxaldialdehyd. Die dem Triose-Reduktion strukturell entsprechende Carbonsäure, oft Dioxy-akrylsäure genannt,



dürfte von FENTON, JONES³ und WILKS⁴ aus Glycerinsäure gewonnen worden sein. allerdings ist es noch nicht ganz sicher, welche der möglichen isomeren Formen entsteht. Die isomeren Oxy-brenztraubensäure hat E. CHARGAFF⁵ aus der entsprechenden Bromverbindung dargestellt.

Über die bisher nicht untersuchte Oxydation des Triose-Reduktions durch Hydroperoxyd gibt folgender Versuch Anhaltspunkte.

Versuch a

Reaktionslösung: Triose-Reduktion 44 mg (0,5 mMol) 20 ml Wasser
Hydroperoxyd 0,11 ml (1,0 mMol)

In 16 Stunden bei 20° gebildet BaCO₃ 33,6 mg entspr. 0,17 mMol CO₂.

Nach Abschluss der Reaktion verbrauchte die Reaktionslösung 7,5 ml eine 0,093 n. Natronlauge, entspr. 0,7 mMol einbasische Säure. Nach der Destillation dieser Lösung enthielt das Destillat 0,37 mMol einbasische Säure.

Nach der Reaktion verbrauchte die Lösung 26,5 ml einer 0,0046 n. Lösung von Dichlorphenol-indophenol (TR-Lös.), entsprechend 0,24 mMol unverändertem Triose-Reduktion.

1 Mol Triose-Reduktion + 2 Mole Hydroperoxyd bilden

0,34 Mole CO₂

0,18 Mole nicht destillierbare Säure

0,74 Mole destillierbare 1-bas. Säure.

Unverändert blieben 0,48 Mole Triose-Reduktion.

¹ Siehe hierzu auch F. LYNEN, sowie F. LIPMANN, Nobelvorlesung 1954; ferner C. MARTINS, Lieb. Ann. 561, 227 (1948).

² R. A. PETERS, Proc. Roy. Soc. B 139, 143 (1952). — Siehe auch H. WEIL-MALHERBE, Nature 138, 551 (1936).

³ FENTON u. JONES, Soc. 77, 73 (1900).

⁴ FENTON u. WILKS, Soc. 101, 1570 (1912).

⁵ E. CHARGAFF, J. A.C.S. 1942.

Versuch b mit doppelter Menge Hydroperoxyd

Reaktionslösung: Triose-Redukton 44 mg (0,50 mMole)

Hydroperoxyd 0,22 ml (2,00 mMole)

In 16 Stunden bei 20° geb. BaCO₃ 49,8 mg entspr. 0,25 mMole CO₂.

Nach der Reaktion verbrauchte die Lösung 8,0 ml Natronlauge, entsprechend 0,74 mMole einbas. Säure.

Die Reaktionslösung verbrauchte nach der Reaktion 13,3 ml der TR-Lösung, entsprechend 0,12 mMole Redukton.

1 Mol Redukton + 4 Mole Hydroperoxyd bilden also

0,50 Mol CO₂

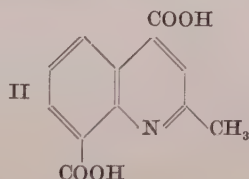
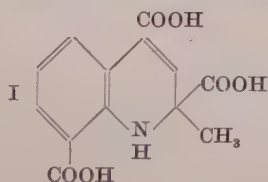
1,24 Mole einbasische Säure.

Mit steigender Menge Hydroperoxyd nimmt also die Menge des entwickelten CO₂ und der gebildeten einbas. Säure zu.

II. Reaktion der Brenztraubensäure mit Anthranilsäure

Beim Erwärmen von 1 Mol Anthranilsäure mit 2 Mol Brenztraubensäure entstehen unter CO₂-Entwicklung 2 Produkte: Das eine, I, wird in Form zitronengelber Prismen vom Schmp 232° m (unkorr.) und der Zusammensetzung C₁₃H₁₁O₆N erhalten. Das andere ist die Dicarbonsäure II.

Die Sbst. I färbt sich im sichtbaren Licht an der Oberfläche innerhalb 1 Min. grün und wird nach 2-stündigem Stehen im Dunkeln wieder gelblich. Auch nach oft wiederholtem Umkrystallisieren behält I die erwähnte Phototropie unverändert bei.



Bei der Prüfung von I mit dem Ehrlichschen Reagens sowie beim Fichtenholz-Test zeigt sich keine Spur von Indol-Reaktion. Erst bei Erwärmen von I auf 85° mit 2-n. Salpetersäure beginnt die Substanz CO₂ abzuspalten. Beim Kochen erfolgt dann eine schnelle und kräftige CO₂-Entwicklung. Die Sbst. I, 1,2-Dihydrochinaldin-2,4,8-tricarbonsäure, geht über in Chinaldin-4,8-dicarbonsäure, Sbst. II. Diese Dicarbonsäure entsteht auch bei der Oxydation von I mit CrO₃ sowie durch Erwärmen derselben in Chinolin auf 100°. Durch Methanolschwefelsäure wird die Tricarbonsäure I an zwei Carboxylgruppen, 4 und 8, methyliert.

Wird die Tricarbonsäure I mit Zinkpulver erhitzt, so bilden sich gut nachweisbare Mengen eines Indol-Derivates. Dieser Übergang des Chinolinringes in den Indolring ist eine auch anderen Chinolin- und Chinaldin-Derivaten eigene Reaktion. Um das durch Erhitzen mit Zn entstehende Produkt hinsichtlich der Indolreaktion näher zu charakterisieren, haben wir unter gleichen Bedingungen mehrere Chinolin-Derivate mit Zn behandelt und mit bekannten Indol-Derivaten verglichen (Tabelle).

Substanz	mg erhitzt	mg gebild. Indol-Derivat	% gebild. Indol-Derivat
8-Oxy-chinolin	20,0	~ 0,002	~ 0,01
Chinaldin-4,8-dicarbonsäure . .	10,0	0,02	0,2
1,2-Dihydro-chinaldin-2,4,8-tricarbonsäure (Subst. I)	10,0	0,18	1,8
Chinaldin-4-carbonsäure	20,4	1,52	7,3

Die verschiedenen Chinolin-Derivate geben bei gleichartigem Erhitzen mit Zinkstaub sehr ungleiche Ausbeuten an Indol-Derivat. Jedenfalls gibt ein reines Chinolin-Derivat (Chinaldin-4-carbonsäure) eine kräftigere Indol-Reaktion als die Subst. I. Für die vor einiger Zeit von EITER¹ vertretene Auffassung, dass die von uns aus Anthranilsäure und Brenztraubensäure synthetisierte Tricarbonsäure nicht ein Chinaldin- sondern ein Indol-Derivat sei, liefert die Indolreaktion der mit Zn erhitzten Tricarbonsäure *keine Stütze*. Da ferner unser Produkt I nicht die geringste Indolreaktion mit dem Ehrlichschen Reagens gibt, spricht nichts gegen unseren, aus früheren Versuchen gezogenen Schluss, dass bei der Erhitzung von Anthranilsäure mit Brenztraubensäure eine Dihydro-chinaldin-tricarbonsäure entsteht, die in der von uns² dargelegten Weise weiteren Umsetzungen zu Chinaldin-Derivaten unterliegt (siehe weiter Kem. Arb. Ny följd B 17, I (1955).

Stockholm, Universitt.

¹ K. EITER, *Experientia* 11, 189 (1955).

² EULER u. H. HASSELQUIST, *Dieses Arkiv* 5, nr 23 (1953).

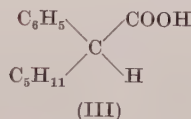
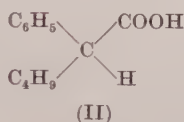
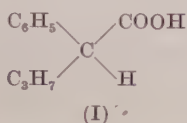
Tryckt den 1 mars 1956

Uppsala 1956. Almqvist & Wiksells Boktryckeri AB

Optical resolution of some α -phenyl fatty acids

By KURT PETTERSSON and GUDRUN WILLDECK

In connection with investigations of the steric relations between some diarylpropionic acids (I) the knowledge of the configurations of α -phenylvaleric (I), α -phenylcaproic (II) and α -phenyloenantic (III) acids has been of particular interest, since these acids constitute possible intermediates for the connection of the diarylpropionic acids with the steric system of D-glyceraldehyde. For that reason, the racemic



acids (I)–(III) were resolved into optical antipodes and the optical activities measured in different solvents. Amides, anilides and S-benzylthiuronium salts were synthesized and in some cases the optical activities were measured in different solvents.

The racemic acids (II) and (III) were prepared by acid hydrolysis of the corresponding nitriles, which were obtained from ethyl phenylcyanoacetate and alkyl halide according to a method described by Wideqvist (2). Resolution of the inactive acids into optical antipodes was performed with (+)- and (–)- α -phenylethylamine, prepared according to a very convenient method, described by Theilacker and Winkler (3).

As early as 1909 Pickard and Yates resolved (I) into optical antipodes by means of *l*-menthylamine (4). Since these authors do not give the temperatures at which the optical activities were measured, it is difficult to compare their values with ours, but the agreement seems to be fairly good. Levene *et al.* (5) have partly resolved (I) and Mislow *et al.* (6) have partly resolved (I) and (II).

None of the optically active acids could be obtained in solid state, and therefore the quasi-racemate method for the determination of configurations could not be applied. However, the amides seem to fulfil the requirements for quasi-racemate formation and have been subjected to thermal analysis. The results will be published in a later paper.

Experimental

Optically inactive α -phenylvaleric acid was kindly supplied by Prof. Arne Fredga.

Amide: The acid was refluxed with excess of thionyl chloride until the evolution of hydrochloric acid ceased (about 15 minutes) and the excess of thionyl

chloride was removed *in vacuo*. The acid chloride was dissolved in benzene and the solution treated with concentrated ammonia. The benzene layer was washed with water, the solvent evaporated and the residue recrystallised from dilute ethanol. The amide was obtained as colourless needles melting at 82° – 84° .

Anilide: The acid chloride, obtained as described above, was dissolved in benzene and the solution treated with aniline. The anilinium chloride was dissolved in water, the benzene layer washed with water and the benzene evaporated. The residue was recrystallised from dilute ethanol and the anilide was obtained as colourless needles melting at 106.5° – 108.5° .

S-Benzylthiuronium salt: The acid was dissolved in ethanol, neutralised with 5N sodium hydroxide and treated with an equivalent amount of S-benzylthiuronium chloride in ethanol. The mixture was boiled for two minutes and then chilled. The sodium chloride was filtered off, the filtrate diluted with water to slight turbidity and then chilled in a refrigerator. The colourless leaves were recrystallised from dilute ethanol. M.p. 142° – 144° .

Ethyl α -phenyl- α -cyanocaproate: 75 g (0.64 moles) of benzyl cyanide, dissolved in 250 ml of diethyl carbonate, was treated with 14.5 g (0.63 moles) of sodium, cut in pieces, and the ethanol formed was removed *in vacuo*. After cooling, 114 g (0.62 moles) of butyl iodide were added and the mixture refluxed for three hours. Water was added and the organic layer was washed with 1N sodium hydroxide, water, 1N sulfuric acid and finally with three portions of water. After drying the mixture was distilled *in vacuo* and the ester collected at 164° – 168° /12 mm Hg.

α -Phenylcaproic acid: Ethyl α -phenyl- α -cyanocaproate was dissolved in ethanol, treated with 35 g of potassium hydroxide in ethanol on a water bath for five minutes, and then allowed to stand at room temperature for three hours. The potassium carbonate was filtered off and washed with ethanol. The ethanol was removed from the filtrate by distillation and the residue was hydrolysed with strong (about 65%) sulfuric acid by boiling for 52 hours. The mixture was diluted with water and the organic acid extracted with ether. After drying the ether was removed and the acid distilled *in vacuo*. α -Phenylcaproic acid was obtained as an almost colourless viscous oil, boiling at 168° – 172° /10 mm Hg. Redistillation gave an oil, boiling at 117° – 120° /0.1 mm Hg. Yield: 108 g or 91 %, based on the butyl iodide used.

124.2 mg of the acid consumed 6.44 ml of 0.1006N NaOH.

$C_{13}H_{16}O_2$: Equiv. weight calc. 192.2 found 191.8.

Amide: Colourless needles. M.p. 95° – 97° .

Anilide: Colourless needles. M.p. 90.5° – 92° .

S-Benzylthiuronium salt: Leaves. M.p. 121.5° – 122.5° .

Ethyl α -phenyl- α -cyanoheptanoate: From 75 g (0.64 moles) of benzyl cyanide, 14.5 g (0.63 moles) of sodium, 250 ml of diethyl carbonate and 100 g (0.66 moles) of amyl bromide as described above for the next lower homologue. The time of reflux was in this case twelve hours. The ester boils at 174° – 178° /12 mm Hg.

α -Phenylloenantic acid: From ethyl phenylcyanoheptanoate as described for phenylcaproic acid. After about three hours boiling with strong sulfuric acid destruction began as indicated by the darkening of the reaction mixture and evolution of vapours. The heating was interrupted and the mixture steam-dis-

Table 1.

Base	(I)	(II)	(III)
Strychnine	Oil	Oil	Oil
Brucine	Oil	Oil	Oil
Quinine	$\pm 0.0^\circ$	Oil	-13°
Quinidine	Oil	Oil	Oil
Cinchonine	$\pm 0.0^\circ$	-8.4°	Oil
Cinchonidine	$\pm 0.0^\circ$	Oil	Oil
<i>l</i> -Ephedrine	-13°	Oil	Oil
(+)- α -Phenylethylamine	-33°	-12°	-37°
(+)-Benzedrine			$+ 2^\circ$

tilled. From the distillate 20 g of the desired acid was obtained by extraction with ether and vacuum distillation. B.p. 178° – $182^\circ/10$ mm Hg. The dark residue from the steam-distillation was extracted with ether, the ether removed and the residue distilled *in vacuo*. 50 g of a colourless viscous oil, boiling at 178° – $182^\circ/10$ mm Hg was obtained. Total yield 70 g or 54 %. Redistillation gave an oil, boiling at 119° – $126^\circ/0.1$ mm Hg, which soon crystallised and melted at 36° – 38° . Recrystallisation from petroleum ether gave a product with the same melting point.

131.1 mg of the acid consumed 6.30 ml of 0.1006*N* NaOH.

$C_{13}H_{15}O_2$: Equiv. weight calc. 206.2 found 206.9.

Amide: Colourless needles. M.p. 100° – 102° .

Anilide: Colourless needles. M.p. 115° – 117° .

S-Benzylthiuronium salt: Colourless leaves. M.p. 114.5° – 115.5° .

Preliminary tests on resolution: 0.001 mole of the racemic acids and 0.001 mole of the optically active bases were dissolved in 10 ml of hot ethanol. The solutions were left at room temperature until crystals or oils were formed or until all solvent had disappeared. The crystals were filtered off, and the acid liberated with dilute sulfuric acid and extracted with ether. The ether was removed, the residue dissolved in ethanol and the optical activity measured in a 2 dm tube. The concentration of the acid was determined volumetrically with sodium hydroxide. The results for the three acids are shown in Table 1.

Optical resolution of α -phenylvaleric acid

(+)- α -Phenylethylamine (–)- α -phenylvalerate: 35.6 g (0.2 moles) of racemic acid and 24.2 g (0.2 moles) of (+)- α -phenylethylamine were dissolved in hot ethanol. After cooling, the crystals were filtered off, dried and weighed. 0.2–0.5 g were set apart, the acid liberated with dilute sulfuric acid, extracted with ether and isolated by evaporation of the solvent. The acid was dissolved in ethanol and the optical activity measured in a 2 dm tube. The concentration of the acid was determined volumetrically with sodium hydroxide. The principal part of the precipitate was recrystallised from ethanol until the liberated acid showed

Table 2.

Crystallisation number	(-)-(I)		(+)-(I)	
	g of salt obtained	$[\alpha]_D^{25}$	g of salt obtained	$[\alpha]_D^{25}$
1	26	-35°	18	+38°
2	22	-48°	15	+50°
3	18	-57°	13	+59°
4	14	-60°	12	+62°
5	13	-62°	10	+63°
6	13	-63°	9	+63°
7	12	-63°	8	+63°

constant optical activity. The pure (+)-phenylethylamine (-)-phenylvalerate was obtained as colourless needles. The progress of the resolution is seen in Table 2.

(-)- α -Phenylethylamine (+)- α -phenylvalerate: The mother liquor from the first crystallisation above was evaporated to dryness and the acid liberated with dilute sulfuric acid and extracted with ether. The ether was removed and the residual acid treated with an equivalent amount of (-)- α -phenylethylamine in hot ethanol. The salt obtained was recrystallised and the optical activity of the liberated acid determined in the same manner as described above for the (-)-acid salt. The results are shown in Table 2.

(+)- α -Phenylvaleric acid: (-)- α -Phenylethylamine (+)- α -phenylvalerate was treated with dilute sulfuric acid and the liberated organic acid extracted with ether. The ether was removed and the (+)-phenylvaleric acid was obtained as an almost colourless viscous oil. Attempts to crystallise the acid were without success.

106.4 mg of the acid required 7.70 ml of 0.0776N NaOH.

$C_{11}H_{14}O_2$: Equiv. weight calc. 178.2 found 178.0.

90.5 mg of the acid was dissolved in absolute ethanol, made up to 10.00 ml and the optical activity measured.

$2\alpha_D^{25} = +1.147^\circ$ $\searrow [\alpha]_D^{25} = +63.4^\circ$ $[M]_D^{25} = +113.0^\circ$

Amide: Colourless crystals. M.p. 106°–108°.

S-Benzylthiuronium salt: Colourless crystals. M.p. 148°–149°.

(-)- α -Phenylvaleric acid: (+)- α -Phenylethylamine (-)- α -phenylvalerate was treated with dilute sulfuric acid and the liberated (-)- α -phenylvaleric acid isolated as described for the (+)-acid. Colourless viscous oil.

104.2 mg of the acid required 7.53 ml of 0.0776N NaOH.

$C_{11}H_{14}O_2$: Equiv. weight calc. 178.2 found 178.3.

Optical activity in homogenous state: $[\alpha]_D^{20} = -76.2^\circ$ $[M]_D^{20} = -135.5^\circ$.

Optical activity in different solvents: Weighed amounts of the acid were dissolved in the given solvent, made up to a volume of 10.00 ml and the optical activity of the solution was measured in a 2 dm tube. In the case of water the

acid was dissolved in 1.0 ml of ethanol, neutralised with 0.0776*N* NaOH and finally made up to 10.00 ml with water. The optical activity was then measured in the usual way. The results are shown in Table 3.

Table 3.

Solvent	mg of acid	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Acetone.	114.1	-2.015°	-88.3°	-157.4°
Benzene	135.5	-2.070°	-76.4°	-136.1°
Acetic acid	126.2	-1.816°	-72.0°	-128.2°
Ether.	101.7	-1.342°	-66.0°	-117.6°
Ethanol.	153.4	-1.963°	-64.0°	-114.0°
Water (ion)	104.2	-0.06°	-2.8°	-5.1°

Amide: Colourless needles. M.p. 106°–108°.

Optical activity in different solvents (*vide ante*) are given in Table 4.

Table 4.

Solvent	mg of amide	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Benzene	79.1	-1.532°	-96.8°	-171.6°
Ether.	83.5	-1.536°	-91.9°	-163.0°
Acetone	80.4	-0.800°	-49.8°	-88.2°
Ethanol.	100.4	-1.003°	-50.0°	-88.5°
Acetic acid	99.7	-0.550°	-27.6°	-48.9°

Anilide: Colourless needles. M.p. 116.5°–117.5°.

Optical activities in different solvents (*vide ante*) are given in Table 5.

Table 5.

Solvent	mg of anilide	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Ether.	103.7	-2.974°	-143.4°	-363.4°
Benzene	88.9	-2.373°	-133.5°	-338.2°
Ethanol	81.2	-1.519°	-93.5°	-237.0°
Acetic acid	93.7	-1.569°	-83.7°	-212.2°
Acetone.	81.0	-1.174°	-72.5°	-183.6°

S-Benzylthiuronium salt: Colourless leaves. M.p. 148°–150°.

Optical resolution of α -phenylcaproic acid

(-)- α -Phenylethylamine (+)- α -phenylcaproate: 19.2 g (0.1 mole) of racemic α -phenylcaproic acid and 12.1 g (0.1 mole) of (-)- α -phenylethylamine were dis-

Table 6.

Crystallisation number	(+)-(II)		(-)-(II)	
	g of salt obtained	$[\alpha]_D^{25}$	g of salt obtained	$[\alpha]_D^{25}$
1	12.5	+33°	8.0	-37°
2	10.5	+49°	6.2	-50°
3	9.6	+55°	5.0	-57°
4	8.9	+63°	4.2	-61°
5	8.5	+63°	3.5	-63°
6	8.2	+63°	3.3	-62°

solved in hot ethanol and the solution allowed to cool. The crystals obtained were recrystallised from ethanol and the resolution followed as described for the resolution of phenylvaleric acid. The optically pure salt formed long, colourless needles. The progress of the resolution is shown in Table 6.

(+)- α -Phenylethylamine (-)- α -phenylcaproate: The mother liquor from the first crystallisation of the (+)-acid salt was evaporated to dryness, the organic acid liberated with dilute sulfuric acid and extracted with ether. The ether was removed and the residual acid treated with an equivalent amount of (+)-phenylethylamine in ethanol. The resolution was carried out as described for phenylvaleric acid. The results are shown in Table 6.

(+)- α -Phenylcaproic acid: (-)- α -Phenylethylamine (+)- α -phenylcaproate was treated with dilute sulfuric acid and the liberated (+)-phenylcaproic acid isolated as described for the optically active phenylvaleric acids. Viscous almost colourless oil.

102.4 mg of the acid consumed 6.88 ml of 0.0776N NaOH.

$C_{12}H_{16}O_2$: Equiv. weight calc. 192.2 found 191.9.

96.7 mg of the acid was dissolved in absolute ethanol and made up to 10.00 ml.

$2\alpha_D^{25} = +1.207^\circ$ $[\alpha]_D^{25} = +62.4^\circ$ $[M]_D^{25} = +120.0^\circ$.

Amide: Colourless waxy crystals. M.p. 92°-93°.

Optical activities in different solvents (*vide ante*) are given in Table 7.

Table 7.

Solvent	mg of amide	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Benzene	106.6	+1.796°	+84.2°	+161.1°
Ether	85.7	+1.395°	+81.4°	+155.6°
Acetone	96.4	+0.871°	+45.2°	+86.4°
Ethanol.	146.0	+1.223°	+41.9°	+80.1°
Acetic acid	115.7	+0.672°	+29.2°	+55.8°

Anilide: Colourless crystals. M.p. 98°-100°.

Optical activities in different solvents (*vide ante*) are shown in Table 8.

Table 8.

Solvent	mg of anilide	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Ether.	100.4	+ 2.674°	+ 133.2°	+ 356.1°
Benzene	107.0	+ 2.644°	+ 123.6°	+ 330.4°
Ethanol.	96.8	+ 1.606°	+ 82.9°	+ 221.8°
Acetic acid	96.4	+ 1.515°	+ 78.6°	+ 210.1°
Acetone	86.9	+ 1.284°	+ 73.9°	+ 197.6°

S-Benzylthiuronium salt: Colourless crystals. M.p. 117.0°–117.5°.

(–)- α -Phenylcaproic acid: From the (+)-phenylethylamine salt as described for the (+)-acid. Almost colourless viscous oil.

111.2 mg of the acid consumed 7.66 ml of 0.0754*N* NaOH.

C₁₃H₁₆O₂: Equiv. weight calc. 192.2 found 192.5.

Optical activity in homogenous state: $[\alpha]_D^{20} = -72.9^\circ$ $[M]_D^{20} = -150.8^\circ$.

Optical activities in different solvents (*vide ante*) are given in Table 9.

Table 9.

Solvent	mg of acid	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Acetone	99.2	– 1.629°	– 82.1°	– 157.8°
Benzene	166.8	– 2.501°	– 74.9°	– 144.1°
Acetic acid	104.3	– 1.502°	– 72.0°	– 138.4°
Ether	147.3	– 1.916°	– 65.0°	– 125.0°
Ethanol.	116.0	– 1.430°	– 61.6°	– 118.5°
Water (ion)	115.1	– 0.154°	– 6.7°	– 12.8°

Optical resolution of α -phenyloenantic acid

(–)- α -Phenylethylamine (+)- α -phenylheptanoate: 20.6 g (0.1 mole) of racemic phenyloenantic acid and 12.1 g (0.1 mole) of (–)- α -phenylethylamine were dissolved in hot ethanol and the solution allowed to cool. The salt obtained was recrystallised from ethanol and the resolution followed as described for phenylvaleric acid. The optically pure salt was obtained as long, colourless needles. Table 10 shows the result of the resolution.

(+)- α -Phenylethylamine (–)- α -phenylheptanoate: The mother liquor from the first crystallisation of the (+)-acid salt was evaporated to dryness and the acid isolated and converted to the (+)- α -phenylethylamine salt as described for the (–)-phenylcaproic acid. The salt was recrystallised and the resolution followed in the usual manner. The results are collected in Table 10.

Table 10.

Crystallisation number	(+)-(III)		(-)-(III)	
	g of salt obtained	$[\alpha]_D^{25}$	g of salt obtained	$[\alpha]_D^{25}$
1	17	+33°	13	-34°
2	15	+43°	11	-53°
3	14	+55°	9.0	-58°
4	12	+59°	7.6	-59°
5	11	+60°	6.5	-60°
6	10	+60°	6.0	-59°

(+)- α -Phenyloenantic acid: From the (-)-phenylethylamine salt as described for the other antipodes. Colourless, highly viscous oil.

108.2 mg of the acid consumed 6.78 ml of 0.0776N NaOH.

$C_{13}H_{18}O_2$: Equiv. weight calc. 206.2 found 205.7.

96.7 mg of the acid was dissolved in absolute ethanol and made up to a volume of 10.00 ml. $2\alpha_D^{25} = +1.162^\circ$ $[\alpha]_D^{25} = +60.2^\circ$ $[M]_D^{25} = +124.1^\circ$.

Amide: Colourless waxy crystals. M.p. 95.5°–96.5°.

Optical activities in different solvents (*vide ante*) are collected in Table 11.

Table 11.

Solvent	mg of amide	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Benzene	127.1	+2.368°	+93.2°	+191.2°
Ether	98.8	+1.832°	+92.7°	+190.2°
Acetone	98.8	+0.938°	+47.5°	+97.4°
Ethanol	96.1	+0.895°	+46.6°	+95.6°
Acetic acid	151.6	+1.187°	+39.2°	+80.3°

Anilide: Colourless crystals. M.p. 98°–100°.

Optical activities in different solvents (*vide ante*) are collected in Table 12.

Table 12.

Solvent	mg of anilide	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Ether	88.4	+2.268°	+128.3°	+361.0°
Benzene	110.0	+2.722°	+123.7°	+348.2°
Ethanol	138.1	+2.245°	+81.3°	+228.7°
Acetic acid	76.8	+1.207°	+78.6°	+221.1°
Acetone	112.4	+1.531°	+68.1°	+191.6°

S-Benzylthiuronium salt: Colourless leaves. M.p. 116.5°–117.5°.

(-)- α -Phenylloenantic acid: Colourless, highly viscous oil, obtained from the (+)- α -phenylethylamine salt in the same manner as described for the other antipodes.

96.1 mg of the acid consumed 6.02 ml of 0.0776N NaOH.

$C_{14}H_{18}O_2$: Equiv. weight calc. 206.2 found 205.7.

Optical activity in homogenous state: $[\alpha]_D^{20} = -65.8^\circ$ $[M]_D^{20} = -135.8^\circ$.

Optical activities in different solvents (*vide ante*) are collected in Table 13.

Table 13.

Solvent	mg of acid	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Acetone.	91.1	-1.416°	-77.7°	-160.3°
Benzene	80.3	-1.152°	-71.7°	-147.9°
Acetic acid	93.8	-1.218°	-64.9°	-133.9°
Ether.	84.0	-1.007°	-59.9°	-123.6°
Ethanol.	106.1	-1.253°	-59.1°	-121.8°
Water (ion)	96.1	-0.177°	-9.2°	-19.1°

Amide: Colourless waxy crystals. M.p. 106° – 108° .

Summary

α -Phenylvaleric, α -phenylcaproic and α -phenylloenantic acids have been resolved into optical antipodes by means of the active forms of α -phenylethylamine. Amides, anilides and S-benzylthiuronium salts of the acids have been synthesized and the optical activities of several of the compounds have been measured in different solvents.

ACKNOWLEDGEMENTS

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Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, and Pharmacia Research Laboratory, Uppsala, November 1955.

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A reaction between silica and some magnesium compounds at room temperature and at $+37^{\circ}\text{C}$

By NILS HAST

With 14 figures in the text

Silica, SiO_2 , in all the forms in which it appears is a very resistant and inactive compound. At room temperature it reacts chemically with only a small number of other compounds. Silica and all silicates dissolve in hydrofluoric acid, forming the gaseous SiF_4 . They are also soluble in melted Na_2CO_3 or K_2CO_3 , forming alkali-silicates soluble in water. In pure water the solubility of silica is low but, depending upon the kind of SiO_2 material and the particle size, some solution takes place in strong alkaline solutions (NaOH or KOH) and also when a water solution of sodium or potassium carbonate containing suspended SiO_2 -particles is heated.

On carrying out investigations concerning a possible transformation of amorphous silicic acid into crystalline cristobalite at room temperature as a result of adding some particular chemical compounds (these investigations will be published as a separate paper), the author has found the following facts:

1. Silica reacts at room temperature with MgO , $\text{Mg}(\text{OH})_2$ and MgCO_3 , forming a silicate, meerschaum, which is very resistant and almost insoluble in water. Other kinds of Mg -compounds, i.e. MgCl_2 and MgSO_4 , although very soluble in water, do not, in analogous experiments, give any silicate reaction which can be seen by an X-ray investigation.

2. In a water suspension of finely ground crystalline quartz the amount of SiO_2 in solution will be stabilized at a low value, if MgO , $\text{Mg}(\text{OH})_2$ or MgCO_3 is added. When adding, besides the MgCO_3 , also a quantity of a calcium salt, i.e. calcium chloride or sulfate, a further decrease of the SiO_2 content, and a more rapid one, takes place.

The principal interest in this investigation (see p. 353–360) is to give rise to a possible way of preventing silicosis in the human lung by using the found silica-combining capacity of MgO , $\text{Mg}(\text{OH})_2$ and MgCO_3 . When using MgO [$\text{Mg}(\text{OH})_2$] parts of these compounds will transform into MgCO_3 when they are brought into the lung atmosphere with its content of CO_2 .

The above silicate reaction takes place when mixing water solutions of SiO_2 and the above Mg -compounds as well as when SiO_2 and the Mg -compounds, both finely ground, are mixed as a paste, or when making a suspension of them in more water. The forming of the silicate sometimes will take place so rapidly that the time of reaction is not more than a few hours.

As an example of this silicate reaction the following experiment may be reported:

Finely ground silica gel (The Coleman and Bell Co., pro analysi) was mixed with MgO (of the type heavy, J. T. Baker Chemical Co.) in the proportions 2:1 by weight and water was added until the consistency was that of a soft paste, the mixture then being stored at $+37^\circ\text{C}$. As early as in $2\frac{1}{2}$ hours the X-ray powder photograph of the mixed mass distinctly showed the appearance of a reaction product. In five hours there were only traces of the MgO-compound lines left in the spectrum, while the compound which was formed gave strong lines. It seems to be a hydrosilicate of Mg, the sepiolite meerschaum (see p. 350 and Fig. 6 and 7).

When mixing silica gel and MgO with higher MgO-content, i.e. $\text{SiO} : \text{MgO} = 1.3 : 1$ in weight, the X-ray lines of MgO did not disappear for some days. When the mixture was more rich in MgO the said lines did not disappear at all, even after the mixture had been stored for a long time at $+37^\circ\text{C}$. However, the excess of MgO would then have been hydrated.

In the X-ray diagram of the Mg-silicate formed there are some strong lines (see Figs. 1-4). The broadening of the lines indicates that the silicate is a very fine crystalline product. If the reaction takes place at room temperature, at $+37^\circ\text{C}$, at a temperature in the vicinity of 100°C or at even higher temperatures (120°C obtained by heating in autoclaves), there do not seem to be any marked differences as to the size of the crystals formed, but the rate of progress of the reaction is increased with increasing temperature.

Experiments have been carried out by mixing finely ground silica gel and MgCO_3 with water to a soft paste. The chemical compound formed is found to be the same (the same X-ray diagram) as when using MgO; however, the silicate reaction takes place much more slowly. At ordinary room temperature the silicate lines in the X-ray spectrum only begin to appear after about two months. When mixing together water solutions of MgCO_3 and SiO_2 , however, a precipitate appears within a few days, this precipitate giving the same X-ray spectrum as results from the MgCO_3 or $\text{MgO} + \text{silica gel}$ mixture described above.

It is of interest to mention, that when mixing MgO or MgCO_3 in quite dry condition with dry silica gel no silicate reaction takes place. It therefore seems probable that the above Mg-silicate is a hydro-silicate or, anyhow, that water is needed for the progress of the reaction. It has also been noticed that by drying a moist preparation of the Mg-silicate there will be some change in the positions of the lines in the X-ray diagram without, however, any broadening of the lines. This may indicate that the silicate found has in some way a zeolitic character. Fig. 2 shows that it is stable up to a temperature of about 700°C (for one hour heating) and that at a higher temperature it transforms into another silicate. The change in the X-ray line positions by heating up to temperatures of 400, 600, 800 and 1000°C can also be seen. It may be added that the temperature in the oven was continuously increased during the heating period, apart from being held at a constant level for one hour at the above temperatures.

When mixing finely ground crystalline quartz and MgO or MgCO_3 in distilled water it is not possible to show by X-ray methods that there is a silicate forming, even after a storage time of many months (at $+37^\circ\text{C}$). However by heating the mixture in an autoclave at 120°C for 24 hours or even less, the X-ray spectra show the formation of the silicate. But it seems to be most likely that already at room temperature a thin layer of Mg-silicate will be formed on to the surfaces of the quartz particles. The layer will increase in thickness during the autoclave heating, so that it becomes possible to observe by X-ray methods. In any case the existence of such

a surface layer consisting of silicates, which are very slightly soluble in water should largely prevent the solution of the quartz particles in water. In fact, the amount of SiO_2 in solution in a water suspension of fine quartz particles decreases almost to zero on the addition of MgO and MgCO_3 to the suspension. These experiments will be described later in this paper.

At first sight it seems to be inconsistent that MgO , Mg(OH)_2 and MgCO_3 , slightly soluble in water (9×10^{-3} and 10^{-1} g per liter) do react with SiO_2 materials forming a silicate, while very soluble salts, i.e. MgCl_2 and MgSO_4 , do not give any noticeable reaction. The latter salts, when in a water solution exhibit an acid reaction, the pH value here found to be 4.2 and 6.5 respectively, while Mg(OH)_2 and MgCO_3 give values as high as 11 and 9.6 respectively. The fact that a salt does not form between silica and MgCl_2 or MgSO_4 seems feasible from the point of view that these Mg-compounds are acid in water solution. The Mg-carbonate and-hydroxide give a basic reaction and salt formation may take place. It may be added, however, that in an experiment in which a mixture of finely ground silicic acid with MgSO_4 and water at $+37^\circ \text{C}$ was held at a constant pH value of 7.5 by the addition of small quantities of H_3N , no silicate seemed to be formed.

The following experiment, R 380, has also been carried out. A very dry paste of silica gel, MgO and water was made and very small particles of this mixture were spread as separate flakes on the bottom of a container in which the CO_2 content of the air could be considerably increased. The reactions taking place were followed by studying the X-ray photographs of the particles. These photographs showed that the presence of free CO_2 -gas around the small particles of SiO_2 and MgO did not prevent the silicate reaction.

X-ray powder photographs of the silicate, formed in a mixture of SiO_2 and MgO or MgCO_3

The mixture of finely ground silica gel and MgO , in the proportions 2:1 as mentioned above (p. 344), was stored at $+37^\circ \text{C}$ and at a relative humidity of 100 %. Samples for an X-ray investigation were taken from the mass at 0, $2\frac{1}{2}$, 5, 10 and 24 hours after the mixing was made. A standard Philips X-ray round camera was used, $d=57.5$ mm. Fig. 1 a-e gives the changes in the X-ray spectra as the silicate formation progresses. In the spectrum from the mass just mixed, Fig. 1 a, only the lines of MgO and the diffuse SiO_2 -halo can be seen. In Fig. 1 b, $2\frac{1}{2}$ hours after mixing, the strongest lines of the Mg-silicate already appear in the spectrum. $2\frac{1}{2}$ hours later all the MgO -lines are quite extinguished. In five hours from mixing the mass the X-ray diagram of the silicate found was strong and well established except for the innermost lines, corresponding to the largest atomplane distances in the structure. It is noteworthy that in none of the spectra of this mixture can any lines of Mg(OH)_2 be seen, although there is plenty of water in the mixture. It seems likely that the silicate formation proceeds more quickly than does the MgO -hydration. Separate experiments have shown that the same finely ground MgO mixed with water gives an X-ray spectrum two hours after mixing in which the Mg(OH)_2 -lines are rather strong and another, three hours later, in which they dominate, traces only of an MgO -diagram being left.

Fig. 3 gives the X-ray photograph of a mixture of silica gel and MgO in the proportions $\text{SiO}_2:\text{MgO}=1.3:1$, which had been stored for 3 months at $+37^\circ \text{C}$ in

a relative humidity of 100 %. This spectrum also shows that there are no traces of MgO, Mg(OH)₂, MgCO₃ left.

Fig. 4 shows the X-ray diagram of a precipitate formed when water solutions of silicic acid and MgCO₃ are mixed. Fig. 5 a and b represent a paste of silica gel and MgO, mixed in the ratio 1 : 1. The excess of MgO was at least partly dissolved away by suspending this mixed mass (three months old) in water, through which CO₂ was passed for some days. After this procedure the remaining parts of the material were stored in a dry condition for 6 weeks. Fig. 5a is an X-ray diagram of this material. Fig. 5b refers to the same material, after heating for 12 hours at a temperature of 120° C in an autoclave. No marked differences can be seen between the X-ray photographs in Fig. 5a and b, nor between these and Fig. 1-4.

In Fig. 4 there are sharp lines from crystalline quartz. These lines are not to be seen in any of the other diagrams. It is of interest to mention that the crystalline quartz has been formed here from a silica gel solution, the concentration of which is only 30 mg SiO₂ per liter, i.e. a value far below the known saturation value of silicic acid in water (about 170 mg per liter at a temperature of 37° C).

Fig. 6 shows an X-ray diagram of the mineral meerschaum¹ compared to that shown in Fig. 5b. The linear marks between both halves indicate lines in these diagrams which seem to correspond with each other. The agreement can also be seen in Fig. 7, which is a photometer record of the blackening intensity in both the X-ray films of Fig. 6. The existence of some differences might be expected, as the silicate product precipitated has a very fine crystalline form. Consequently, the surfaces of its crystallites are very large and it may be possible that the small differences in the diagrams are related to this fact. In Fig. 7 there are 7 corresponding blackening maxima in both the films. The maximum d of the meerschaum does not appear in the artificial Mg-silicate. All other maxima of appreciable intensity do exist in both the photometer records. Also, the relative intensities of the maxima are in rather good agreement in the two diagrams. It may be added (see M. H. Hey, *Chemical Index of Minerals*, Brit. Mus. 1950, p. 88) that in the natural Mg-hydrosilicates sepiolite the Mg : Si ratio is said to vary from 9:11 to 1:2, and this adds to the difficulty of the identification problem. An approximate analysis of the silicate prepared by mixing solutions of SiO₂ and MgCO₃, gave a Mg:Si ratio of about 0.6 and a small amount of crystal water. It appears that the Mg-silicate found seems most likely to be the sepiolite meerschaum.

Electron micrographs of the Mg-silicate formed

Fig. 8a is a micrograph taken with a Siegbahn-Schönander electron microscope ($\times 12,500$). It shows the silicate formed, when water solutions of silicic acid and MgCO₃ are mixed, the same preparation as in Fig. 4 (the X-ray investigation). Fig. 8b shows a detail in a higher magnification ($\times 62,000$). The structure of the Mg-silicate seems to be homogeneous without any large crystals. Fig. 8b in the higher magnification shows the ultimate fine structure.

A suspension was made of finely ground rock crystal in a pure, centrifuged solution of MgCO₃. Fig. 9 shows some particles from this suspension after three weeks. Fig. 10 shows rock crystal particles which have been stored in water for the same time. The preparations in Figs. 9 and 10 have been care-

¹ The author wants to thank Professor F. Wickman, Naturhistoriska Riksmuseet Stockholm for the meerschaum specimens.

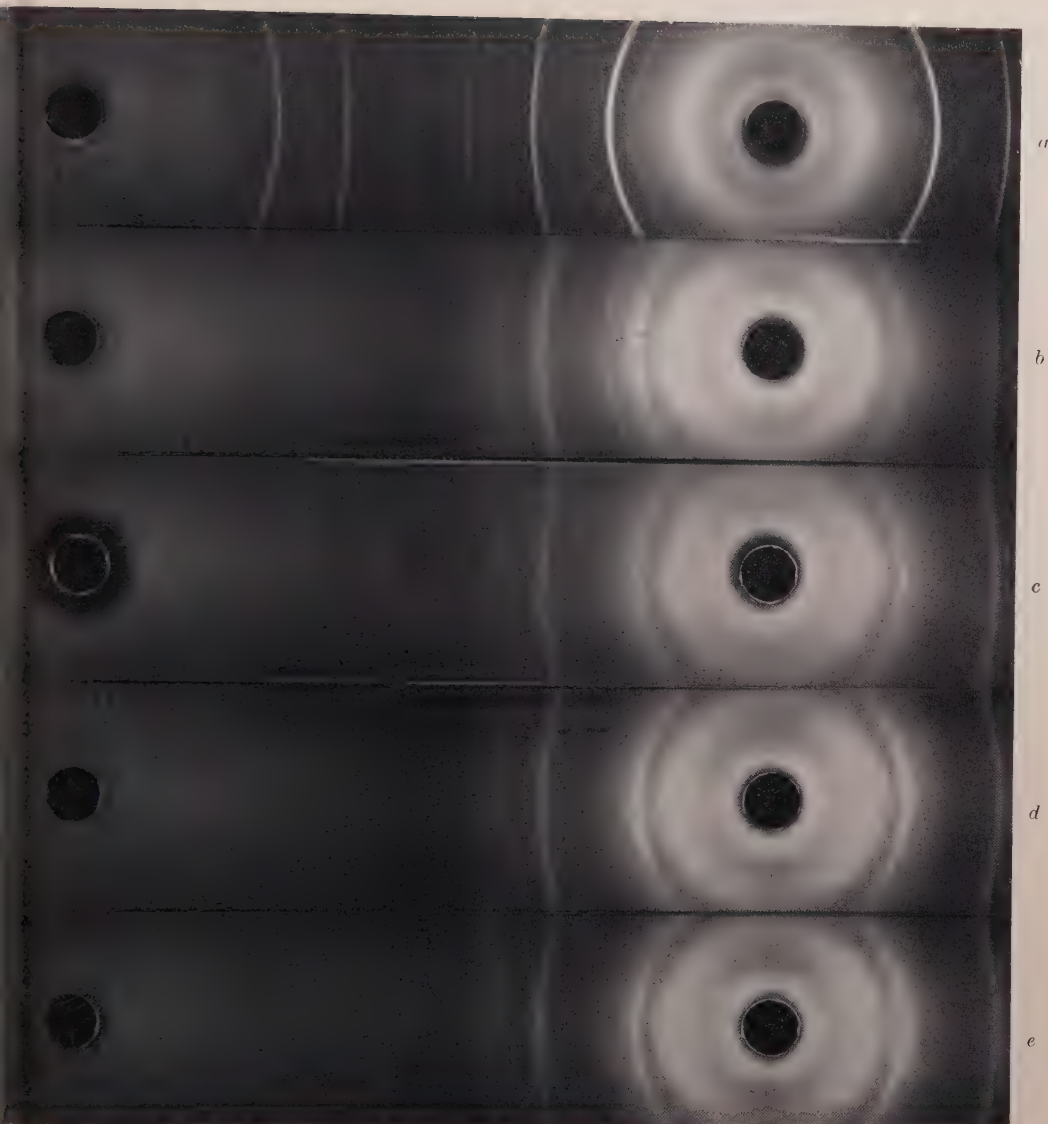


Fig. 1. A mixture of finely ground silica gel and MgO in distilled water, $\text{SiO}_2:\text{MgO}=2:1$ in weight (dry mixture), stored at 37°C in a relative humidity of 100 %. The X-ray diagrams *a-e* show the proceeding silicate formation in 0, $2\frac{1}{2}$, 5, 10 and 24 hours resp. after the mixing was made. Diagram *a* represents the mass just mixed: only the lines of MgO and the diffuse SiO_2 halo can be seen. Diagram *b*, $2\frac{1}{2}$ hours later, shows that the silicate formation has already taken place and that almost all the MgO content is consumed. There are no differences between *c*, *d* and *e* except that some faint outer lines are slightly more visible in *e* than in *c*.

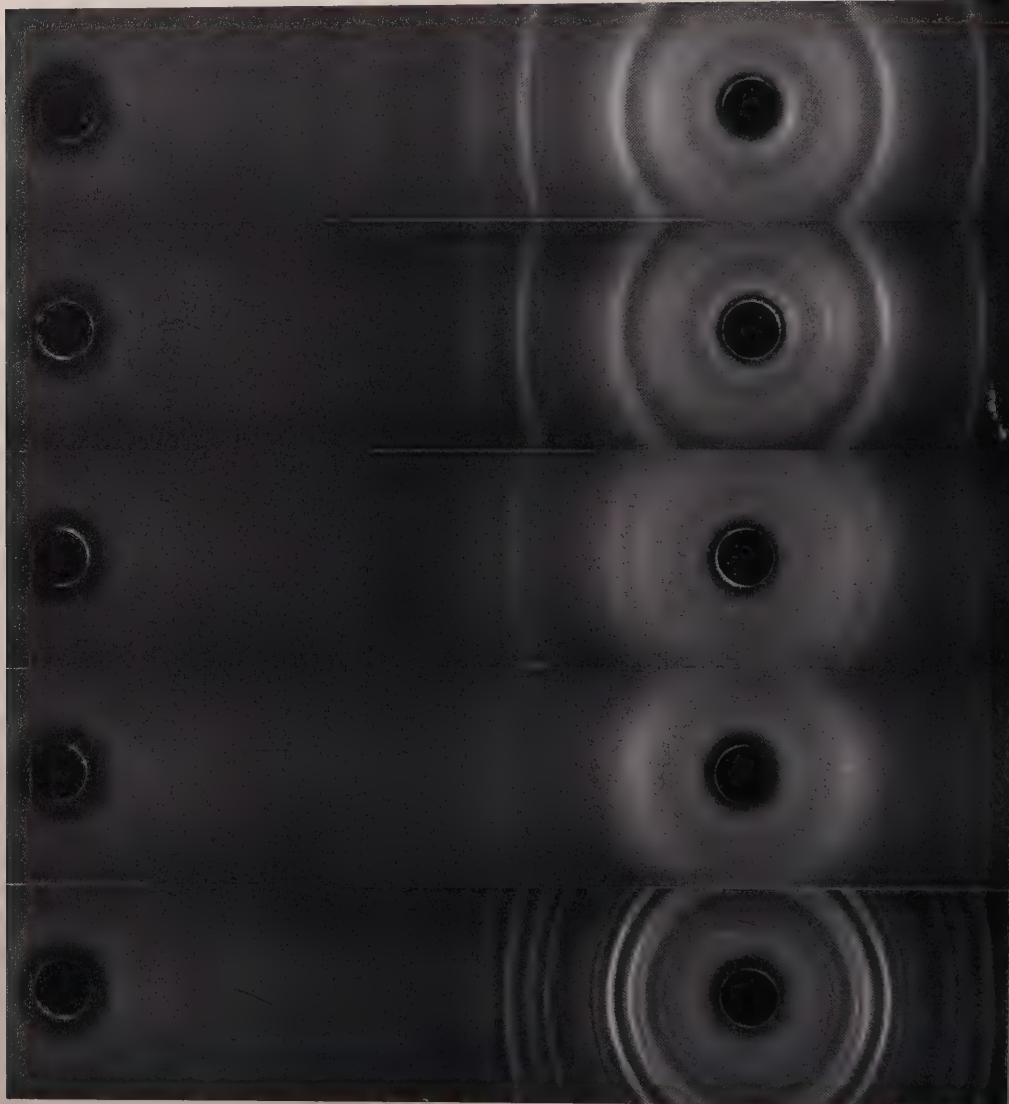


Fig. 2. The figure shows that the actual Mg-silicate is stable up to a temperature of about 700°C . The temperature in the oven was continuously increased, from 20° (a) to 1000° (e), but was held at a constant level for one hour, at 400° , 600° , 800° and 1000°C —diagrams b, c, d and e respectively.

fully washed in distilled water before the electron microscopical preparation was made. It is apparent that the quartz particles in Fig. 9 are covered with a silicate layer, meerschaum as mentioned above, while the quartz particles in Fig. 10 are unaffected. This silicate layer impedes the solution of the SiO_2 particles in the water, as found in the suspension experiment R 367, p. 352.



Fig. 3. The X-ray diagram of a mixture of silica gel and MgO; $\text{SiO}_2 : \text{MgO} = 1.3 : 1$ in weight, stored for 3 months at $+ 37^\circ\text{C}$ in a relative humidity of 100 %. No lines of MgO, $\text{Mg}(\text{OH})_2$ or MgCO_3 are left.

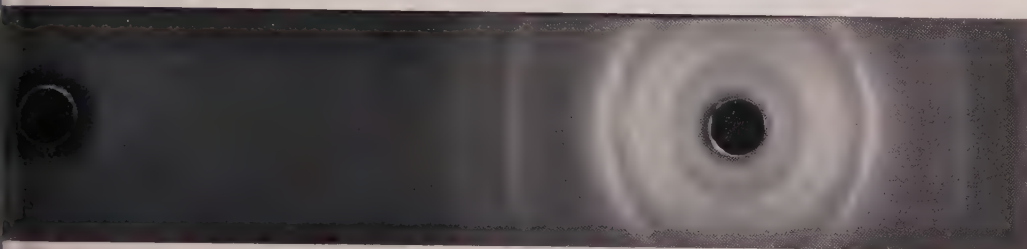
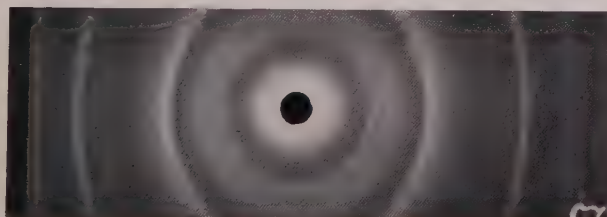


Fig. 4. X-ray diagram of a precipitate formed when a water solution of silicic acid and another solution of MgCO_3 are mixed.



a



b

Fig. 5 a. The X-ray diagram of a mixture of finely ground silica gel + MgO (1 : 1 in weight), the excess of MgO being extracted by suspending the mixture (3 months old) in a large quantity of water through which CO_2 was passed for some days.

Fig. 5 b. The same preparation as in Fig. 5 a, but heated for 12 hours at $+ 120^\circ\text{C}$ in an autoclave. There seem to be no differences between diagrams 5 a and 5 b.

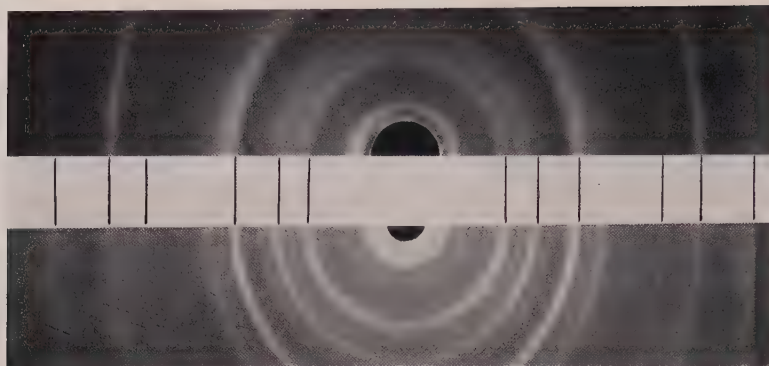


Fig. 6. The X-ray diagram in Fig. 5 b compared with the diagram of the natural sepiolite mineral meerschaum (lower picture). Lines which seem to correspond are connected.

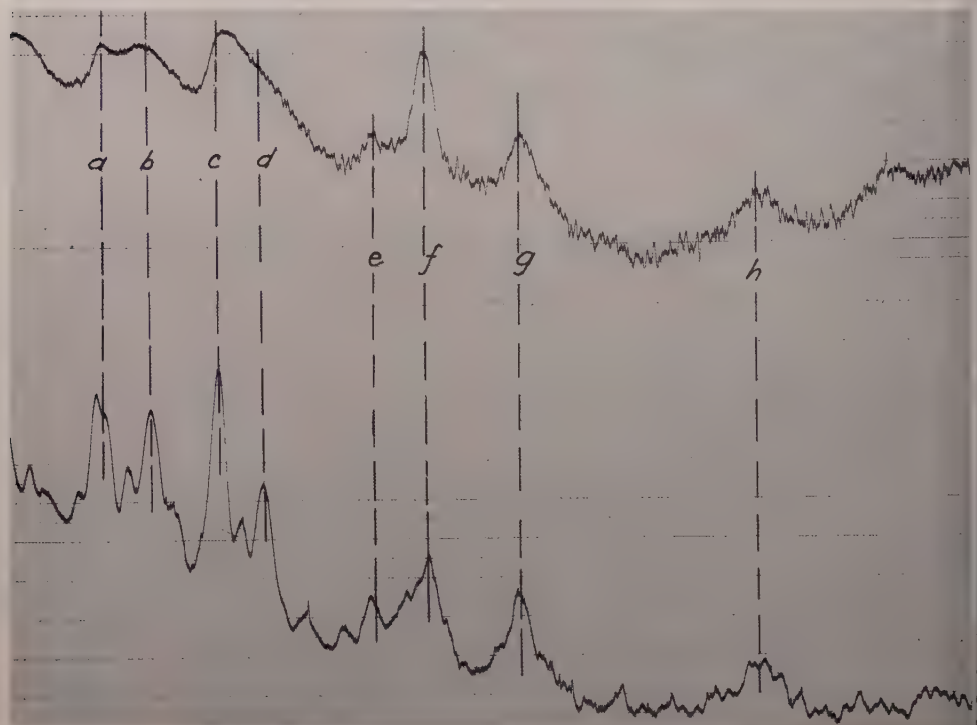


Fig. 7. A photometer record of the blackening intensity in both the X-ray films of Fig. 6. Upper curve relates to the artificial product, lower curve to the mineral meerschaum. There are seven corresponding maxima, the relative intensities of which being in a rather good agreement in the two diagrams. The maximum *d* of the natural meerschaum, however, does not appear in the artificial product; this line possibly relates to some impurity of the mineral sample. This photometer record has been taken with an apparatus at the Institute of Inorganic and Physical Chemistry, University of Stockholm, a facility for which the author is indebted to Professor Arne Ölander.

A suspension was also made of finely ground crystalline quartz and MgO ($\text{MgO}:\text{SiO}_2 = 0.4:1$ in weight). Twenty hours later a small amount of the solution was diluted with a large quantity of distilled water. A preparation for the electron microscope was made from a drop of the diluted suspension. Fig. 11 shows particles in this drop. The SiO_2 particles are all covered with hydrated MgO and, as stated above, nearest to the quartz surfaces a Mg-silicate layer. It is of interest to note that there is a marked agglomeration tendency between the particles of MgO, $\text{Mg}(\text{OH})_2$ and SiO_2 . In fact the particles seem to combine in a way much alike that known from particles in a set cement material.

Investigations concerning the solubility of SiO_2 , when either finely ground silica gel or crystalline quartz is suspended in water to which $\text{MgO}[\text{Mg}(\text{OH})_2]$ or MgCO_3 is added

In the following the results from two series of experiments will be reported, one series identified as R 365 a-d with 4 suspensions containing (besides MgO or MgCO_3) finely ground silica gel or crystalline quartz, the other R 367 with 7 suspensions containing finely ground crystalline quartz with MgO or MgCO_3 in different proportions.

Suspension experiment R 365

Material used in this experiment:

1. Silica as silica gel supplied by the Coleman and Bell Co., containing about 20% water, having a HF residue of 0.4 per cent and passing a No. 200 Sieve (pro analysi).
2. Silica as finely ground crystalline quartz (rock crystal, 99.8 per cent SiO_2).
3. MgO of the heavy type (J. T. Baker Chemical Co.).

Four different suspensions were made, each one containing 250 ml distilled water, and were stored in closed bottles of polythene at a temperature of $+37^\circ\text{C}$. Every second day the bottles were shaken by hand for about 1 minute.

After 25 days the suspensions R 365 a-d were centrifuged and the amounts of SiO_2 in solution in the suspension water measured. The molybdate method was used.¹ The results are to be found in column I in Table 1.

Table 1.

	SiO_2 in solution (mg/l)		
	I	II	III
R 365 a: 5 g silica gel + 1 g MgO	103	100	60
b: 5 g „ „ + 2.5 g „	48	90	56
c: 5 g „ „ + 4 g „	5.0	1.0	7.2
d: 5 g finely ground quartz + 2.5 g MgO .	3.4	0.0	4.5

¹ These measurements were made at the water laboratory of AB. Kemibrån Stockholm.

After this centrifuging 250 ml of distilled water was added to the solid residue in each bottle. Six days later another centrifuging was made and the SiO₂-content of the suspension water was again measured—column II. Distilled water, 250 ml per bottle, was again added to the solid residue and shaken as before. 50 days later these third suspensions were centrifuged and the SiO₂-content in solution measured. The values found are reported in column III.

In (a), column I, the amount of MgO added does not seem to have any retarding effect on the SiO₂-content in solution. The solubility values are about the same as when suspending silica gel alone. It seems likely that the amount of MgO added has been quite consumed in the silicate formation and free silica is left. In (b), MgO gives some retarding effect, probably as a result of the silicate formation. In (c), however, with 4 g MgO added to the suspension, the SiO₂ in solution has decreased to a very low value, probably indicating that the silicic acid is consumed in the Mg-silicate formation. 5 mg SiO₂ per liter may be the solubility of the silicate itself.

What has been said about the figures in Table 1, column I, holds good also for columns II and III. The long suspension time in column III gives some change in the SiO₂ content in a and b, but the solubility values are still high in these cases. In the suspensions c and d the retarding effect upon the SiO₂-solubility in water still exists.

Suspension experiment R 367

2.5 g finely ground crystalline quartz (rock crystal, 99.8 % SiO₂) were suspended in 200 ml distilled water in a bottle of polythene. 7 such suspensions were prepared. To three of them, R 367 b-d, MgCO₃ was added in varying amounts, to another three, R 367 b₁-d₁, MgO was added also in varying amounts and to one of the suspensions no addition at all was made.

R 367 a 2.5 g finely ground crystalline quartz

b	same	+0.05 g MgCO ₃
c	same	+0.25 „ „
d	same	+1.25 „ „
b ₁	same	+0.05 „ MgO
c ₁	same	+0.25 „ „
d ₁	same	+1.25 „ „

All these suspensions were stored at a temperature of +37° C and agitated for about 1 minute a day and no fresh suspension water was added throughout the experiment. The amount of SiO₂ in solution was measured after 3½, 9½ and 16½ days from the preparation of the suspensions. The weight of the suspended quartz particles was initially 1.25 % of that of the suspension water but this percentage increased after each sampling, as some of the suspension water had to be removed for each determination of the SiO₂-content in solution. Thus the experiment referred to the retarding effect on the SiO₂-solubility in more concentrated suspensions than the initial one. However, information relating to these concentrations are of no less interest. The experiment has been repeated with larger quantities of suspensions, the concentration values in this way being kept rather constant throughout the experiment.

The curves of Fig. 12 and Fig. 13 show that the solubility of quartz particles in water is highly retarded by the addition of MgCO_3 or MgO to the suspension. Even as small an amount as 10 % of the weight of the quartz particles is almost completely effective in this respect, the SiO_2 -content in solution being rapidly stabilized at a low value of about 10 mg per liter. When adding MgO or MgCO_3 to the extent of 50 % of the quartz weight the remaining concentrations of SiO_2 are found to be only 4 and 8 mg per liter respectively. It may be added that the main part of this SiO_2 -content seems to arise from the solution of the Mg-silicate formed and not from free SiO_2 in solution. Even smaller additions, 2 % MgO or MgCO_3 , have an appreciable influence and this effect became more marked as the concentration of the suspension was increased in this experiment by drawing off quantities of the suspension water for the measuring of the SiO_2 -content in solution, as noted above.

The experiment R 365 d represents a suspension in water of finely ground quartz (the weight of the particles being 2 % of that of the water) and 50 % MgO , i.e. half the weight of the quartz. The storage time was 25 days at a temperature of 37°C . The SiO_2 -content in water solution, as seen in Table 1, was, in this experiment, found to be 3.4 mg per liter, this value agreeing rather well with that obtained from curve d in Fig. 13.

By comparing the series R 365 and R 367 it is obvious that the reactions between MgO , MgCO_3 and silica gel and quartz particles takes place in different ways. The ultimate fine structure of silica gel is very porous and this fact makes it possible for the Mg-ions to react with this kind of SiO_2 -material all over its extremely large gel-surfaces. On the other hand, it seems likely that the silicate reactions with the crystalline quartz particles will mainly take place only at the surfaces of the particles or in their near vicinity. A reference is here made to the electron micrograph Fig. 9 which shows the existence of such a surface layer of a Mg-silicate around the SiO_2 particles and the structure of this layer. Therefore it is not surprising that much smaller amounts of MgCO_3 or MgO have a retarding effect on the SiO_2 -solubility when mixed with crystalline quartz particles than when mixed with silica gel.

Suspension experiment R 395

The experiment R 367 shows that by adding MgCO_3 to a water suspension of finely ground crystalline quartz, there will be a marked decrease in the amount of SiO_2 in solution. When adding, besides the MgCO_3 , a quantity of a calcium salt, i.e. calcium sulfate or chloride, the SiO_2 content decreases even more, almost to zero, as can be seen in Fig. 14.

Some possible applications of the combination of SiO_2 with certain magnesium compounds

From a mineralogic point of view it seems to be of interest to note that SiO_2 with MgO or MgCO_3 at room temperature forms sepiolite (meerschaum) as the above experiments have shown. It would therefore seem possible that also the natural deposits of this mineral can have been formed at room temperature and not only under hydrothermal conditions.

From a medical point of view the possibility of converting free SiO_2 to a silicate at ordinary room temperature without using compounds which may be injurious

to the human body is of great interest. It has been stated that the serious industrial disease of silicosis is found only under such working conditions in which the air is polluted by dust particles consisting of *free silica* and not in cases in which the particles are silicate minerals. There is one exception known, that of asbestos dust, which gives rise to asbestosis, a different form of silicosis. The present investigations, from which it appears that silicic acid combines with MgO [Mg(OH)₂] or MgCO₃ at room temperature, forming a silicate which is very resistant even in air with a high CO₂ concentration, suggest the possibility of using MgO or MgCO₃ to combat silicosis. Such a method should impede the onset of pathological silicosis changes in lungs not previously affected and may also be of value in cases where the silicosis changes are going on.

The air exhausted from the human lung has a CO₂-content of about 4 per cent. The fundamental requirements for a material which will prevent silicosis must then be, firstly, that the SiO₂-combining compound will not be rendered inactive by the presence of CO₂ in the lung and, secondly, that the silicate formed must itself be resistant to the influence of CO₂ in the air around it. These conditions seem to be fulfilled by MgO[Mg(OH)₂] and Mg-carbonate, which form a silicate, when they come into contact with silica. Further, as was shown above (p. 345), this silicate forms in the presence of a CO₂ atmosphere, even when a mixture of MgO and silica is dispersed as very fine particles.

For a long time it has been well known that silicosis does not occur among workers in some mines, even though free silica is found amongst the mine minerals. It has been stated that in such mines there will always be found calcium minerals, particularly CaCO₃. This very marked circumstance has given rise to the idea of protecting the workers in mines where the silicosis hazard exists by giving them every day inhalations of air, containing a finely dispersed Ca-salt. The most extensive use of this practice has been in German mines; however, the results obtained there do not appear to come up to expectations. The medical experts on the Silicosis Committee of the Swedish Mine Association have been studying the German results and give their opinion as follows:

"The Germans, who have been using the calcium treatment for many years—especially by using fine powdered Ca-salts of different kinds and having the mine workers inhale this dust, and also in recent years by using a fine dispersion of Ca-salt in water solution in the air to be inhaled—cannot statistically show any silicosis-preventing effect of this calcium treatment. Neither have X-ray investigations of the workers shown any positive results."

The fact that the calcium treatment has no effect in the prevention of silicosis, in spite of what is known in practice about the effect of calcium carbonate in mine minerals, may be given the following explanation in view of what has been said above as to the reaction with silica of some Mg-compounds.

In natural deposits of calcium minerals the Ca-compound will very seldom be in a quite pure condition. As a rule there are Mg-ions present to some extent, being mixed with the Ca-ions. The explanation of the non-silicosis state in mines containing free silica and e.g. calcium carbonate may be the presence of Mg-ions as an impurity in the Ca-deposits, these Mg-ions giving the silicosis preventing effect. Ca-salts used for the calcium treatment of the German mine workers are rather pure chemically and this would then explain the lack of any preventative effect.

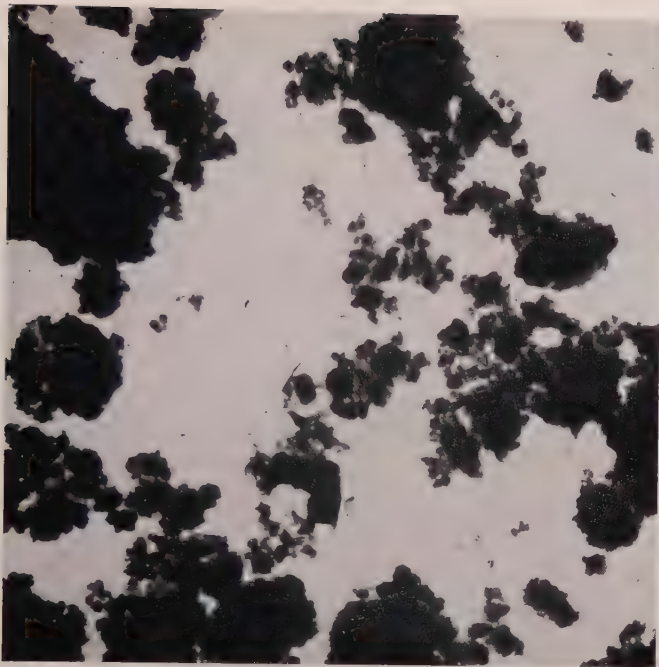


Fig. 8 a. The silicate formed, when water solutions of silicic acid and MgCO_3 are mixed.
 $\times 12,500$.

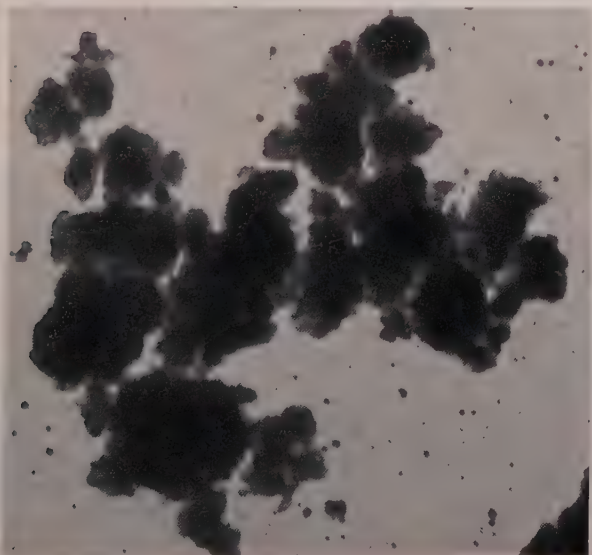


Fig. 8 b. A detail of Fig. 8 a in a higher magnification showing the ultimate fine structure of the
Mg-silicate. $\times 62,000$.



Fig. 9. Finely ground crystalline quartz, stored for three weeks ($+37^\circ\text{C}$) in a pure water solution of MgCO_3 . The SiO_2 particles are entirely covered with a reaction product, probably meerschaum. $\times 50\,000$.

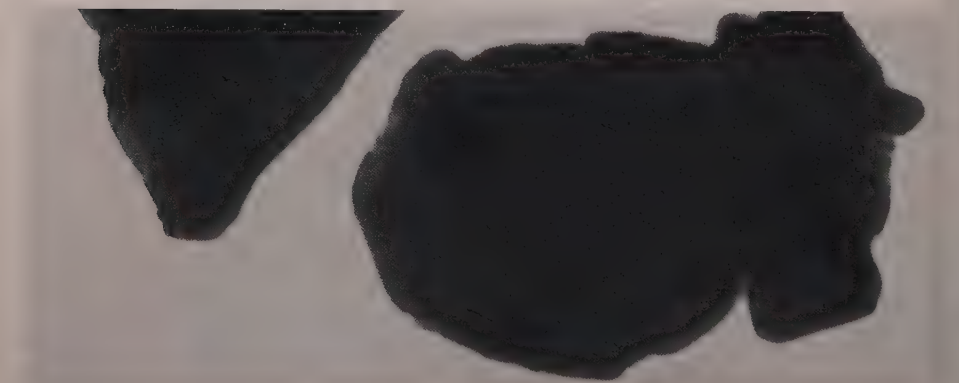


Fig. 10. Finely ground crystalline quartz of the same kind as in Fig. 9, stored in distilled water for four weeks. No reaction product of the same kind is visible. $\times 50,000$.



Fig. 11. Finely ground crystalline quartz and MgO (40 % of the quartz weight) are suspended in distilled water. The electron micrographs show that all the quartz particles are covered with hydrated MgO (some carbonizing may also have taken place). Nearest to the SiO_2 surfaces a Mg-silicate has formed, impeding the further solution of the quartz particles in the suspension water. As can be seen, there exist no longer any minute particles of SiO_2 or MgO. They are all agglomerated, forming larger particles, the smallest ones 8–10 μ . Magnification 10,000 $\times$, i.e. 5 times lower than in the cases of Figs. 9 and 10.

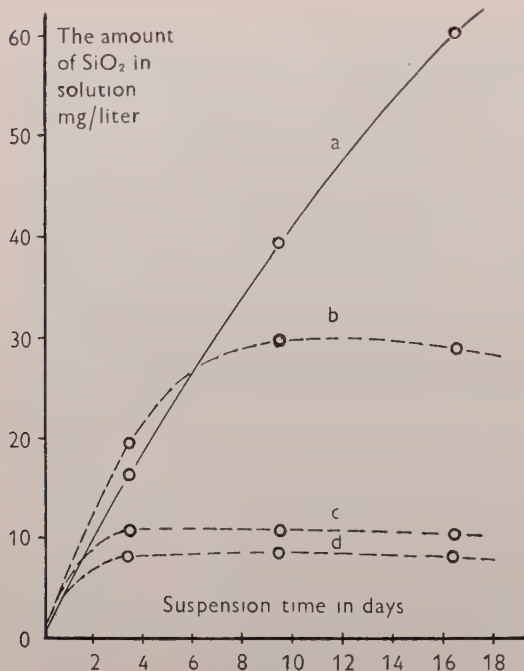
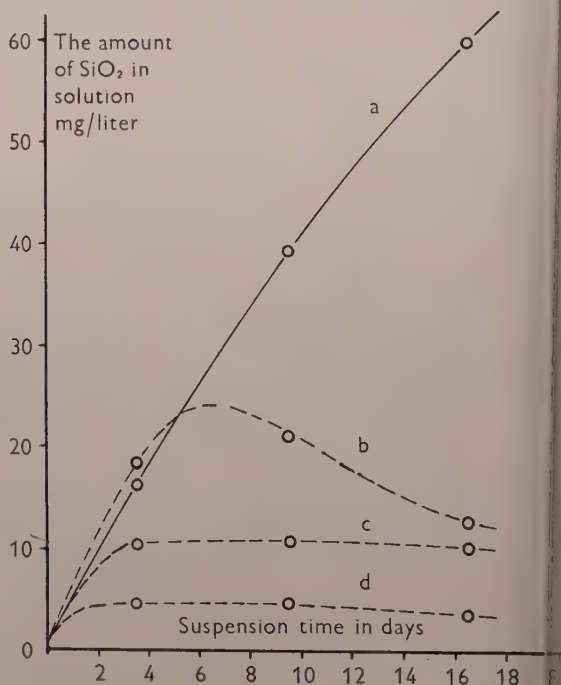


Fig. 12. The solubility of finely ground crystalline quartz in distilled water (+37°C) and the effect on the SiO_2 solubility when adding MgCO_3 in different amounts.

- Curve a A suspension of 2.5 g quartz in 200 ml water
 b Same + $\text{MgCO}_3 = 2\%$ of the quartz weight
 c Same + $\text{MgCO}_3 = 10\%$ of the quartz weight
 d Same + $\text{MgCO}_3 = 50\%$ of the quartz weight.

Fig. 13. The solubility of finely ground crystalline quartz in distilled water (+37°C) and the effect on the SiO_2 solubility when adding MgO in different amounts.

- Curve a A suspension of 2.5 g quartz in 200 ml water
 b Same + $\text{MgO} = 2\%$ of the quartz weight
 c Same + $\text{MgO} = 10\%$ of the quartz weight
 d Same + $\text{MgO} = 50\%$ of the quartz weight



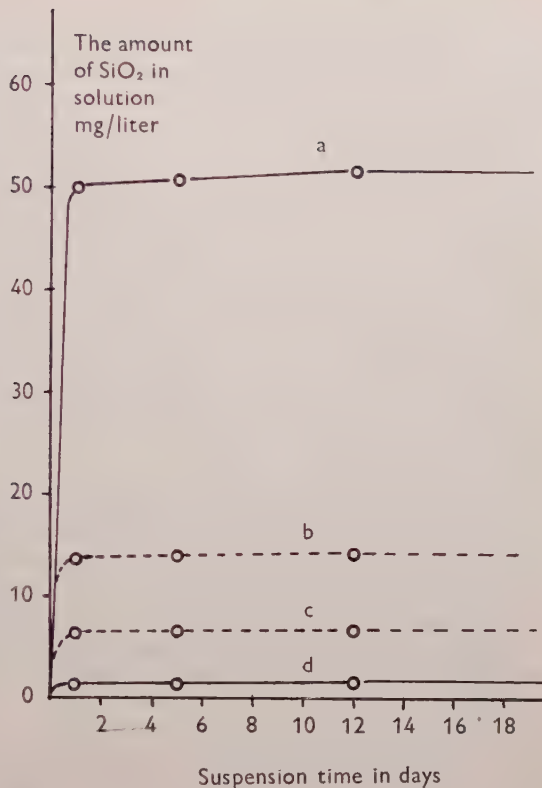


Fig. 14. The solubility of freshly ground crystalline quartz in distilled water (37°C). The effect on the SiO_2 solubility when adding MgCO_3 or $\text{MgCO}_3 + \text{CaSO}_4 \cdot \frac{1}{2} \text{H}_2\text{O}$ to the suspension.

Curve a A suspension of 12 g quartz in 300 ml water

b Same + 5 g MgCO_3

c Same + 5 g MgCO_3 + 1 g $\text{CaSO}_4 \cdot \frac{1}{2} \text{H}_2\text{O}$

d Same + 5 g MgCO_3 + 5 g $\text{CaSO}_4 \cdot \frac{1}{2} \text{H}_2\text{O}$

A remarkable agglomeration effect

As can be seen in Fig. 11 the SiO_2 and MgO particles in a suspension of finely ground quartz and MgO in water are highly agglomerated. Practically no separate fine particles of these compounds can be seen on the whole membrane, which supports the preparation in the electron microscope. All particles are assembled in flakes, apparently with some mechanical strength. The flakes shown in Fig. 11 are the smallest ones to be seen in the preparation and about 10μ in diameter. There will be no such agglomeration when SiO_2 -particles only are suspended in water for the same time.

As an additional experiment, particles of SiO_2 and MgO were suspended in air in a vessel. 15 and 30 minutes after the suspension was made membranes for electron microscopical studies of the particle agglomeration were placed inside the vessel. The micrographs show that particles settled on the membrane are agglomerated. MgCO_3 instead of MgO gives almost the same effect.

In fact, this agglomeration effect of SiO_2 with MgO or MgCO_3 may in itself be of value for combatting silicosis. It is well known that the finest quartz dust is the most dangerous one. Particles $< 1 \mu$ in diameter are very active. As a rule particles $> 5 \mu$ in diameter are too large to pass into the lung alveoli. Such particles are exhausted. When SiO_2 dust agglomerates by using MgO or

MgCO₃ suspended in the drilling water or the mine air, the number of small dangerous particles of silica in the air to be breathed must be greatly reduced. Most of the agglomerated particles will be harmless to the human body as they will grow too large to pass into the lung alveoli. The agglomerated and therefore heavy particles will settle rather quickly and in this way the polluted air may be cleaned. This phenomenon offers one means of preventing silicosis, while the chemical reactions pointed out here constitute another protection.

The only way to discover whether the silica-combining capacity of MgO [Mg(OH)₂], MgCO₃ or some similar substance, as suggested here, will give rise to a possible way of preventing silicosis is to make experiments directly, firstly on animals and, if these experiments give a positive result, on human subjects. It can be mentioned that the inhalation of small amounts of dust particles consisting of the Mg-compounds mentioned does not seem to be injurious to the human body¹ and their solubility in water is low, as is probably also their solubility in the body fluids. Further, no injurious effects have been observed to afflict stone cutters dealing with MgCO₃ material. At any rate, the harmlessness for the human body of the Mg salts mentioned must be proved in separate experiments.

The practical investigations regarding the possible use of the said Mg-compounds as a substance preventing silicosis will be made at the National Institute of Public Health in Stockholm. Before the results of these experiments are available, it is impossible to decide whether the method constitutes any valuable contribution to the silicosis question. The nature of silicosis or the fundamental question of why it appears in the human body, is a very complicated problem and there are certainly many unknown factors, some of which may be of extreme importance to the question.

The X-ray investigation has been carried out in the Division of Building Technic of the Royal Institute of Technology, Stockholm. The micrographs shown have been taken with a Siegbahn-Schönander electron microscope. Ing. Arne Rosenqvist has assisted me in carrying out the investigation.

¹ See P. Drinker and T. Hatch in their book "Industrial Dust" 1954.

Spectrochemical studies on disulphides

IV. The structure of some polysulphides

By LENNART SCHOTTE

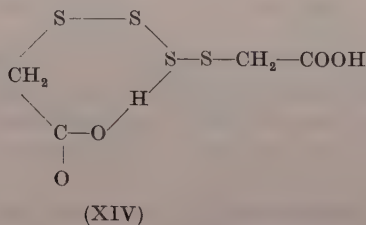
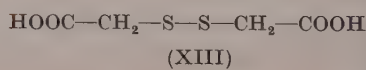
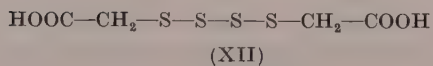
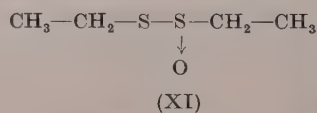
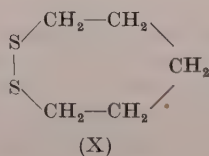
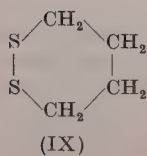
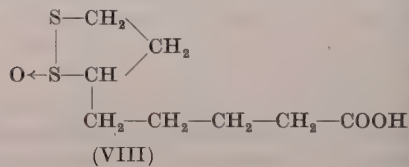
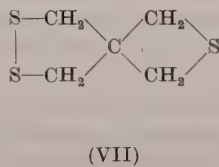
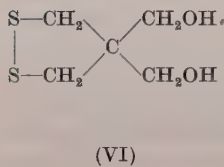
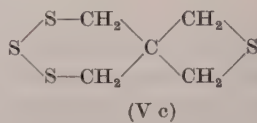
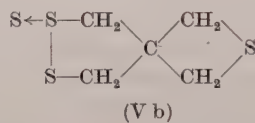
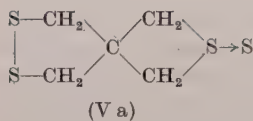
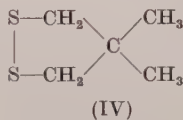
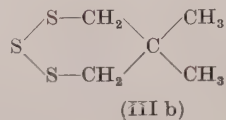
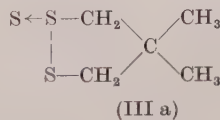
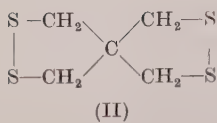
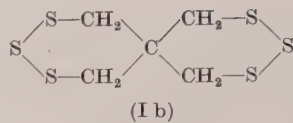
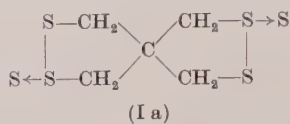
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In the preceding paper of this series an account was given of the spectrochemical behaviour of some 4-substituted 1,2-dithiolanes (1). The substances investigated were first prepared by BACKER and co-workers (2, 3, 4) and in most cases obtained from related trisulphides through a desulphurization procedure. The trisulphides were synthesized from different halogenides in reaction with alkali polysulphides. As the intermediate polysulphides lose one sulphur atom very easily, BACKER assigned to them a structure with coordinately linked sulphur. In view of later work (e.g. 5, 6, 7) it is known, however, that arguments based only on chemical data do not imply unequivocal evidence against an unbranched chain structure. The comprehensive report of Foss (5) about the prevalence of unbranched sulphur chains in polysulphides and polythionic compounds strongly emphasizes the improbability of coordinated sulphur-sulphur bonds. Structure investigations are best made by physico-chemical methods.

On treating pentaerythrityl tetrabromide with excess of sodium tetrasulphide in alcoholic solution a hexasulphide compound is obtained, which was characterized as 2,7-dithio-2,3,7,8-tetrathia-5-spiro-nonane¹ (Ia) (2). The asymmetric isomer demanded by this symmetrical structure was regarded as unlikely since (I) does not give an addition derivative with mercuric chloride. If (I) is treated with a desulphurization reagent one or two sulphur atoms may be removed depending upon whether potassium sulphide or copper is used. The present author was not able to isolate the pentasulphide quite pure but obtained the related tetrasulphide (II) according to BACKER's descriptions. The spectral properties of this substance are reported in (1). No addition product with mercuric chloride is described for the pentasulphide; the tetrasulphide (II) adds only one HgCl_2 per molecule. Therefore the arguments about the structure of (I) are somewhat dubious. No alternative formula with unbranched sulphur chains is discussed.

Analogous to the reaction described, 2,2-dimethyl-1,3-dibromopropane in reaction with sodium tetrasulphide gave a trisulphide (3). A coordinated structure (IIIa) was proposed. Eliminating one sulphur atom, 4,4-dimethyl-1,2-dithiolane (IV) is obtained, the spectral behaviour of which is also discussed in (1).

¹ The numbering is not in accordance with *Ch. A.*



Another polysulphide of spirane structure has been prepared from pentaerythrityl tetrabromide and potassium disulphide (4). BACKER and EVENHUIS discussed two formulae (Va and Vb) both with branched sulphur chain. The decision was made in favour of the structure (Va), according to BACKER's nomenclature, 2-thio-2,6,7-trithia-4-spirooctane.¹ The evidence is, however, obviously questionable. It seems based only upon the fact that the same compound is obtained when potassium disulphide, prepared in solution by saturating an alcoholic solution of potassium hydroxide with hydrogen sulphide and adding a calculated amount of sulphur, is used as when pure sodium disulphide is dissolved in alcohol and then reacted with the halogenide. As the reaction is performed at reflux temperature, we may, however, expect some dismutation of the disulphide. Thus it seems of no importance which method of preparation that is used. From these chemical data no conclusions about the structure (V) can be drawn.

In addition it will be noted that in a few cases no stable trisulphides can be isolated. Thus, 1,1-bis-bromomethyl-cyclohexane and sodium tetrasulphide gave a mixture of tri- and disulphide, the latter in excess (3). Furthermore, dibromopentaerythritol and sodium tetrasulphide (or disulphide) in alcohol-water gave 4,4-bis-hydroxymethyl-1,2-dithiolane (VI) (3), which was also investigated in (1).

Before a more detailed discussion of the polysulphide structures is given, it may be emphasized that molecular weight determinations have proved the monomeric form of the compounds in question in accordance with the assumption of BACKER. The excellent experimental work of BACKER and his co-workers have furthermore given examples of reactions with halogenides of neopentyl type, where no rearrangements occur. This is confirmed by spectral and chemical analyses of the 1,2-dithiolane derivatives (1, 2, 3, 4), and the reason is surely to be found in the symmetrical structure of the halogenides. Moreover, it is noted that in most cases the 1,2-dithiolane derivatives cannot be obtained with alkali disulphide in good yield. Better results are obtained with tetrasulphides.

The spectrochemical behaviour of the three stable compounds (I), (III) and (V) has now been investigated. The formulae proposed by BACKER have been questioned by FOSS (5), who remarks that the chemical evidence reported by BACKER and EVENHUIS (4) for (V) in support of a branched structure (Va) may equally well be true for the unbranched structure (Vc). In Fig. 1 the ultraviolet absorption curve of (I) in chloroform as solvent is shown. The curve is characterized by high intensity absorption, especially if compared with the related 1,2-dithiolane derivative (II) (1), and a maximum at 267 $m\mu$. Analogous behaviour is found for (III) with alcohol as solvent (Fig. 1). Here the absorption peak is obtained at 265 $m\mu$ and the intensity is lowered to an amount, which corresponds well to the existence of two trisulphide functions in (I) and only one in (III). Fig. 2 shows the corresponding UV absorption of (V). It resembles rather closely that of (III), having also a maximum at 265 $m\mu$. For comparison the spectrum of diethyl trisulphide is also given in Fig. 1. It is seen that a distinct point of inflexion is developed in the 260 $m\mu$ region, where the maxima reported above occur. It seems highly probable to the author that diethyl trisulphide has a straight chain structure, as detailed investigations by diffraction methods have revealed unbranched sulphur chains for dimethyl trisulphide (8) and β,β' -bis-iodoethyl trisulphide (9, 10). In this case it is difficult to interpret the synthesis (from ethane thiol and thionyl chloride; (11)) as indicating a coordinative structure. The

¹ The numbering is not in accordance with *Ch. A.*

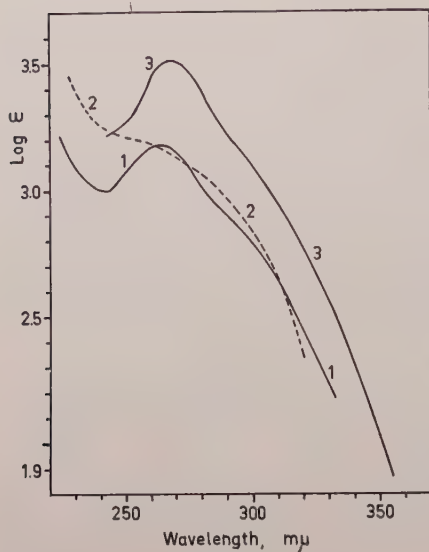


Fig. 1.

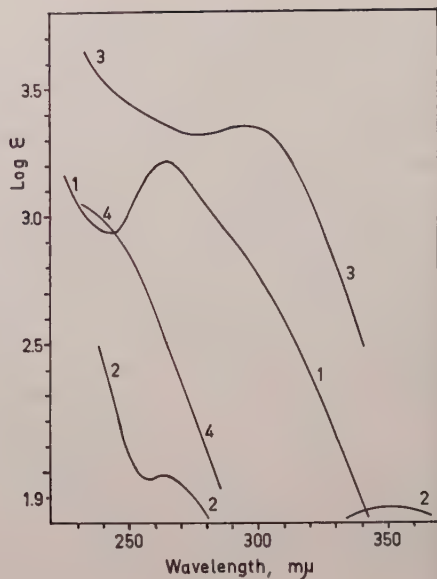


Fig. 2.

Fig. 1. UV absorption spectra of: (1) (III) in alcoholic solution; (2) diethyl trisulphide (solvent: alcohol) (dashed curve); (3) (I) in chloroform solution.

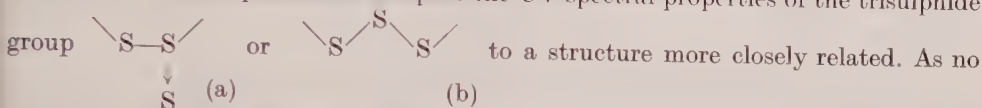
Fig. 2. UV absorption spectra of: (1) (V); (2) 2,6,7-trithia-(3,4)-spirooctane; (3) tetrathio-di-glycolic acid; (4) dithio-di-glycolic acid. Solvent: Alcohol.

spectrum of di-*n*-hexadecyl trisulphide (prepared from the mercaptan and sulphur dichloride) is reported by BAER and CARMACK (12), the general appearance of which seems to be in agreement with that of diethyl trisulphide.

It seems justified to discuss more thoroughly the UV spectrum of (V). If the structure (Va) proposed by BACKER and EVENHUIS is correct, we may regard the spirane as composed of a 1,2-dithiolane ring and a sulphur coordinated thiacyclobutane ring with one carbon atom in common. Comparing the ultraviolet absorption of the desulphurized 1,2-dithiolane derivative (VII), which is partly given again in Fig. 2, to that of (V), we conclude that the structure (Va) cannot be correct, as the influence of the 1,2-dithiolane ring should then be clearly noticeable. Its characteristic absorption in the related (VII) is found near 350 $m\mu$, certainly with comparatively low intensity but nevertheless sufficiently high for a contribution to the extinction above the limits of error in the determinations. The absorption curve of (V) does not show any irregularities in the wavelength region around 350 $m\mu$. Therefore, either (Vb) or (Vc) is valid, and thus the structure problem reduces in common for (I), (III) and (V) to determining whether the sulphur chains are branched or straight.

For a further comment on the ultraviolet absorption spectra of the polysulphides we first note the close similarity of the curves, which makes it probable that the maxima can be ascribed to the same sulphide function. A comparison has already been made with the behaviour of diethyl trisulphide, which is furthermore related to that of

diethyl tetrasulphide (13) and di-*n*-hexadecyl tetrasulphide (12). KOCH and co-workers have presented strong evidence for an unbranched sulphur chain in diethyl tetrasulphide (13) by showing that the ultraviolet absorption curve is of the same spectral type as those of different compounds with known linear structure such as dialkyl disulphides, diphenyl disulphide, disulphur dichloride (14) and sulphur. However, it would be most important to compare the UV spectral properties of the trisulphide



compound with an established structure (a) is to be found, the corresponding sulphoxide was taken as a model. Whereas a sulphoxide of monosulphides in general does not exhibit any characteristic specific UV absorption, maxima are found for saturated aliphatic thiolsulphinates, the frequencies of which vary somewhat with the solvent. The selective absorption is assigned to the system $\begin{array}{c} \diagup \text{S} \diagdown \\ | \\ \text{O} \end{array}$ as a unit.

BACKER and KLOOSTERZIEL (15) have examined a number of thiolsulphinates and in alcoholic solution a high intensity maximum is found at $246 \text{ m}\mu$. In hexane the same substance has its absorption top at $260 \text{ m}\mu$, a difference which is best explained by the influence of hydrogen bonds on the sulphoxide group in the alcoholic solution. The occurrence of hydrogen bonds for sulphoxides in certain solvents has been confirmed by infrared measurements (16). In the case of the trisulphides no influence by the solvent on the specific absorption frequency exists, which has been found for (V) measured in alcohol, chloroform and 2,2,4-trimethyl-*n*-pentane. Thus, it is justified to compare the spectral properties of the thiolsulphinates in hexane with those of the trisulphides. The value of such a comparison is further increased by the fact that 6,8-thioctic acid monoxide (VIII), a five-membered cyclic thiolsulphinat closely related to the trisulphide structures proposed by BACKER, has in alcoholic solution an UV absorption maximum at $244 \text{ m}\mu$ (17), indicating similar spectral properties for the ring as for the non-cyclic substances. The wavelengths to be compared are thus $260 \text{ m}\mu$ for the thiolsulphinates and $265 \text{ m}\mu$ for the polysulphides, a difference which surely is too small to account for the structural change from sulphoxide to coordinated sulphur. The absence of a greater bathochromic shift indicates an alternative structure for the polysulphides. It is certainly true that the extent of conjugation is more extensive for thiolsulphinates than for the corresponding sulphur derivatives but on the other hand the effect of the easier excitable electron pairs of the sulphur atoms seems predominant.

The alternative polysulphide structures with unbranched sulphur chains have the formulae (Ib), (IIIb) and (Vc) resp. That means a structure with a 1,2,3-trithiane ring in the molecule. The unsubstituted system is not known and attempts at synthesis using BACKER's methods have failed. The presence of substituents seems to stabilize the structure. However, it should be possible to deduce roughly the spectrochemical properties of such a compound, if comparison is drawn to the UV behaviour of the six- and seven-membered cyclic disulphides (17). 1,2-Dithiane (IX) exhibits a maximum near $290 \text{ m}\mu$, whereas 4,8-thioctic acid derived from 1,2-dithiepane (X) shows a top near $260 \text{ m}\mu$. Studies of molecular models seem to indicate that in the seven-membered disulphide atomic arrangements are permitted

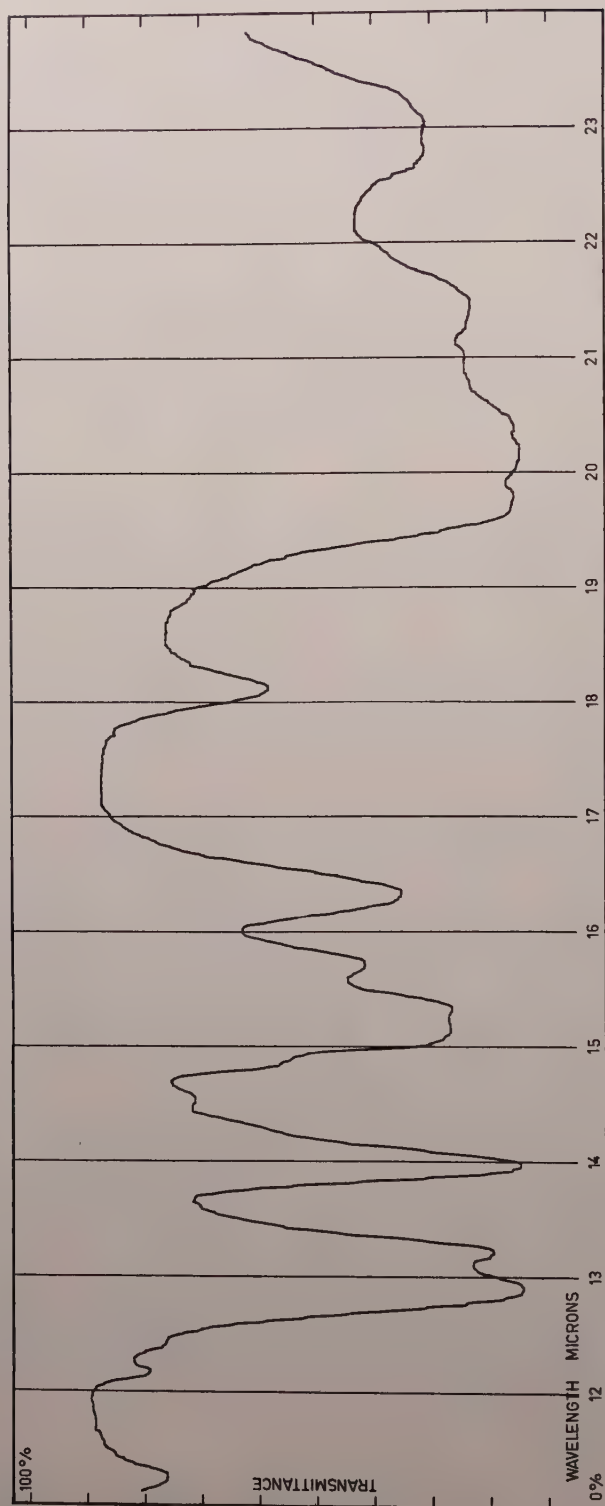


Fig. 3. IR absorption curve of ethyl ethanethiol-sulphinatate (liquid phase) in the KBr prism region.

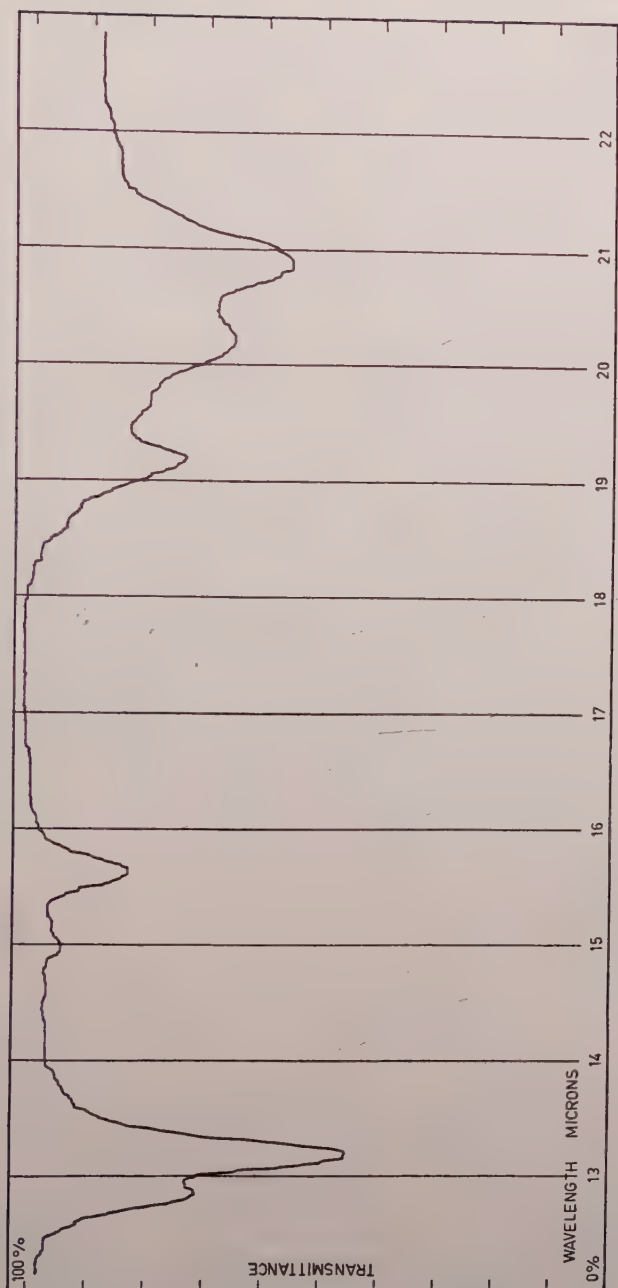


Fig. 4. IR absorption curve of diethyl trisulphide (liquid phase) in the KBr prism region.

which do not imply any significant deviation from the energetically favoured skew configuration of the S-S group. An analogous conclusion for 1,2,3-trithiane seems probable. The 1,2-dithiane structure on the other hand, is more compact, and it is likely that small differences from the S-S properties of a non-cyclic system exist. In any case we must expect an increased repulsion between non-bonding p-electron pairs of the two sulphur atoms with excitation of the disulphide by less energy as a consequence. The bathochromic shift from about $250\text{ m}\mu$ for linear disulphides to near $290\text{ m}\mu$ for the 1,2-dithiane corresponds well to this view. This suggests that an absorption maximum for a 1,2,3-trithiane derivative may be expected at a wavelength below $290\text{ m}\mu$, probably near $260\text{ m}\mu$, which characterized the seven-membered ring disulphide. Moreover, the extension of the sulphur chain from two to three atoms causes increased absorption intensity and probably also a small bathochromic shift due to the repulsive effect of the electrons of the additional sulphur atom. This qualitative deduction thus renders it possible to assign the characteristic ultraviolet absorption to an unbranched polysulphide structure, but the results are not quite unequivocal.

Extending this spectrochemical investigation into the infrared region we find that no examinations with the view to decide between branched and unbranched sulphur chain structures are known. Raman spectra have, however, been useful in this respect (18, 19). Thus, the Raman frequencies of some tri- and tetrasulphides are reported to resemble those of elementary sulphur, whereas no bonds were found in the region to be expected for a coordinative sulphur-sulphur double bond (20). Even if these observations can be applied in our study, we must remember that the spirane structures complicate considerably the spectra with several structural vibrations. Our conclusions should therefore be drawn with care, and the present author simply prefers to make a comparison between the IR spectra of related substances with subsequent interpretation. The IR absorption behaviour of a coordinated sulphur-sulphur bond is certainly not known but we could perhaps get an idea about it from the knowledge of the sulphoxide group. This linkage is known to exhibit specific absorption of high intensity at $1060\text{--}1040\text{ cm}^{-1}$ ($9.4\text{--}9.6\text{ }\mu$). The same type of absorption is now found for ethyl ethanethiol-sulphinat (XI), which has been examined in both the sodium chloride and the potassium bromide prism region. The most characteristic detail in the spectrum for the NaCl region is the broad band around $9.4\text{ }\mu$. Even at longer wavelengths intense absorption occurs as seen from Fig. 3. The wavelengths for both the regions are collected in Table 1. Here it is also of value to note that sodium thiosulphate shows very intense and broad bands in the region $9\text{--}10\text{ }\mu$ (21). It may be concluded from the above that the differences in mode of vibration for a coordinated sulphur-sulphur bond and a normal linkage of disulphide type indicate from the spectral point of view that the branched chain might cause absorption bands of higher intensity and at somewhat lower wavelengths than those in general assigned to the S-S stretchings. The spectra of diethyl trisulphide and diethyl tetrasulphide have been recorded (Table 1) and the part belonging to the potassium bromide prism region is shown in Figs. 4 and 5 resp. These two spectra of polysulphides with highly probable straight chain structures have been compared with data of elementary sulphur and diethyl disulphide and proved to be the same type in accordance with what can be expected from analogous structures. If, on the other hand, Figs. 4 and 5 are compared to Fig. 6, which gives the IR spectra between $11\text{--}24\text{ }\mu$ for (I), we can again confirm a remarkable similarity especially in the region $19\text{--}21\text{ }\mu$, within which the S-S stretching frequency is to be

Table 1. The infrared absorption bands of ethyl ethanethiol-sulphinate and some linear polysulphides.

b = broad; vs = very strong; s = strong; m = medium; w = weak; sh = shoulder; e = emulsion peak.

Ethyl ethanethiol-sulphinate ¹			Diethyl trisulphide ²			Diethyl tetrasulphide ²			Tetrathio-di-glycolic acid ³		
Wave-length in μ	Wave-number in cm^{-1}	Charac-ter of the band	Wave-length in μ	Wave-number in cm^{-1}	Charac-ter of the band	Wave-length in μ	Wave-number in cm^{-1}	Charac-ter of the band	Wave-length in μ	Wave-number in cm^{-1}	Charac-ter of the band
2.32	4310	w	3.2	3125	sh	3.38	2959	vs	2.93	3413	w
2.40	4167	w	3.45	2899	vs	3.44	2907	vs	3.16	3165	m
2.54	3937	w	c. 6.3	c. 1587	sh b	6.92	1445	vs	3.35	2985	m
2.92	3425	vs	6.90	1449	vs	7.05	1418	s	3.75	2667	w
3.45	2899	vs	7.05	1418	vs	7.27	1376	s	4.00	2500	w
4.15	2410	w	7.28	1374	vs	7.54	1326	w	5.55	1802	w
4.3	2326	sh	7.50	1333	s	7.85	1274	sh	5.75	1739	vs
4.5	2222	sh	7.84	1276	s	7.99	1252	vs	5.94	1684	vs
4.65	2151	w	8.00	1250	vs	8.68	1152	w	7.00	1429	s
5.20	1923	w	8.77	1140	w	8.85	1130	m	7.16	1397	m
5.80	1724	vs	9.56	1046	s	9.54	1048	s	7.26	1377	w
6.10	1639	w	9.75	1026	sh	9.74	1027	w	7.87	1271	s
6.23	1605	w	10.36	965	vs	10.35	966	s	7.96	1256	s
6.30	1587	w	12.85	778	m	12.9	775	sh	8.35	1198	m
6.90	1449	vs	13.19	758	s	13.20	758	s	8.88	1126	s
7.0	1429	sh	14.98	668	w	14.25	702	w b	8.95	1117	s
7.27	1376	s	15.62	640	w	15.60	641	w	11.0	909	w b
7.60	1316	s	19.20	521	m	16.50	606	w	12.30	813	m
7.95	1258	vs	20.2	495	m b	18.1	552	w b	c. 12.8	c. 781	sh
8.42	1188	w	20.87	479	m	20.3	493	sh	14.75	678	m
8.53	1172	w				20.85	480	s	15.17	659	m
8.88	1126	s							17.20	581	m
c. 9.4	c. 1064	vs b							17.62	568	m
9.75	1026	sh							20.25	494	m
10.36	965	s							21.08	474	m
11.26	888	m							23.11	433	m
12.17	822	w							23.85	419	w
12.4	806	sh									
12.90	775	vs									
13.22	756	vs									
13.97	716	vs									
14.5	690	sh									
15.2	c. 658	vs b									
15.72	636	s									
16.35	612	s									
18.12	552	m									
19.8	505	vs b									
20.2	c. 495	vs b									
21.4	c. 467	vs b									
22.9	c. 437	s b									

¹ Wavelength region 2–12 μ (NaCl prism); 11–24 μ (KBr prism). Liquid phase.² Wavelength region 3–13 μ (NaCl prism); 12–23 μ (KBr prism). Liquid phase.³ Wavelength region 2–14 μ (NaCl prism); 12–24 μ (KBr prism). KBr phase.

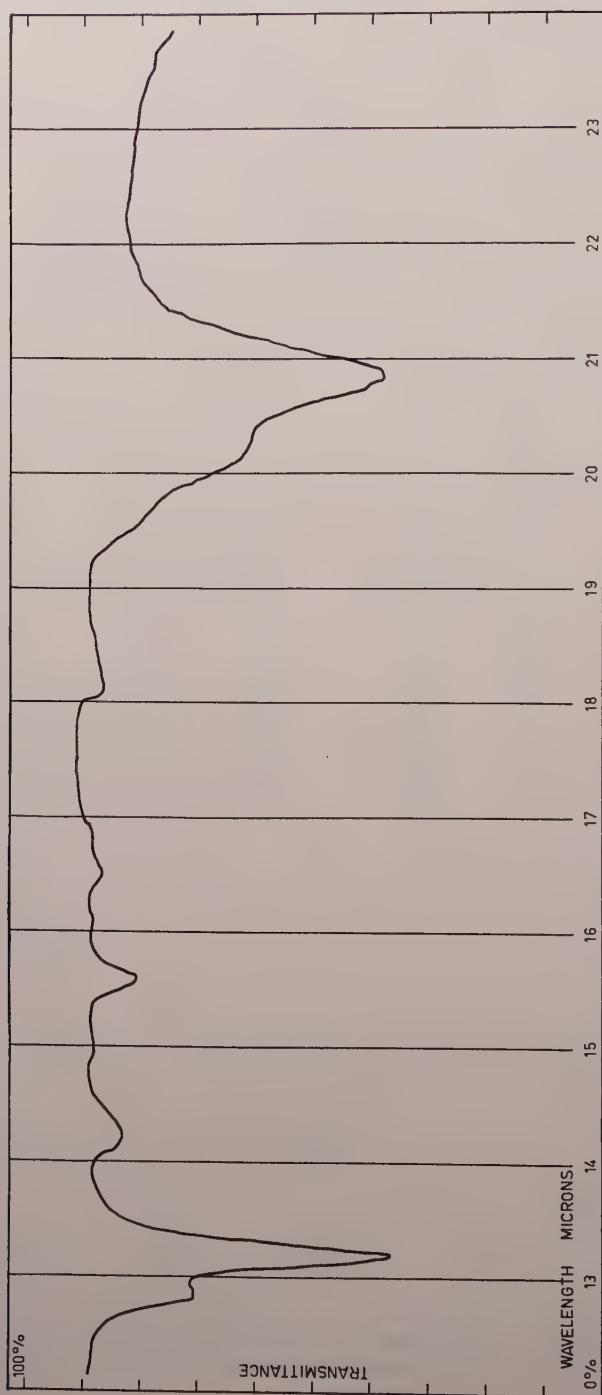


Fig. 5. IR absorption curve of diethyl tetrasulphide (liquid phase) in the KBr prism region.

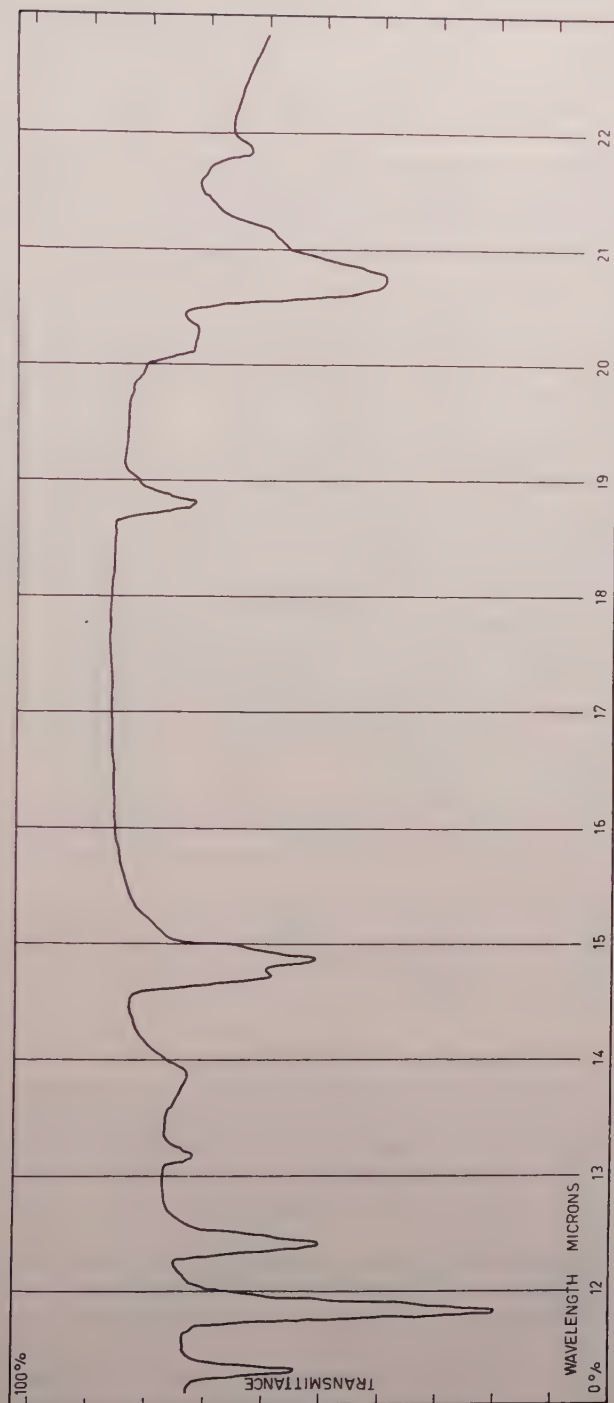


Fig. 6. IR absorption curve of (I) (Nujol emulsion phase) in the KBr prism region.

Table 2. The infrared absorption bands of some cyclic polysulphides.

For abbreviations see Table 1.

(I) ¹			(III) ²			(V) ³		
Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band
7.14	1401	s	3.0	3333	w b	6.85	1460	e
7.19	1391	s	3.40	2941	s	6.94	1441	sh
7.25	1379	s	6.2	1613	w b	7.03	1422	sh
7.68	1302	m	6.80	1471	s	7.13	1403	m
7.77	1287	m	7.08	1412	m	7.23	1383	e
7.90	1266	s	7.17	1395	m	7.86	1272	w
8.03	1245	m	7.23	1383	s	7.95	1258	s
8.20	1220	s	7.32	1366	s	8.20	1220	s
8.60	1163	m	7.80	1282	m	8.45	1183	m
8.67	1153	m	8.08	1238	m	8.55	1170	s
9.35	1070	m	8.72	1147	w	8.95	1117	m
9.42	1062	m	8.87	1127	m	9.50	1053	w
10.06	994	w	8.98	1114	w	9.70	1031	w
11.32	883	m	9.27	1079	w	10.15	985	m
11.82	846	s	9.58	1044	w	11.34	882	s
12.40	806	m	10.12	988	w	12.05	830	m
13.17	759	w	10.44	958	w	12.43	805	m
13.87	721	e	10.64	940	w	13.45	743	m
14.71	680	m	10.87	920	w	13.85	722	m
14.86	673	m	11.55	866	m	14.18	705	m
18.81	532	w	12.12	825	w	14.60	685	w
c. 20.25	c. 494	w b	12.95	772	w	17.88	559	w
20.75	482	m	13.67	732	w	20.10	498	w
21.1	474	sh	14.15	707	m	20.70	483	s
21.85	458	w	20.58	486	m			

¹ Wavelength region 6–13 μ (NaCl prism). KBr phase; 11–23 μ (KBr prism). Nujol emulsion phase.² Wavelength region 3–13 μ (NaCl prism); 11–23 μ (KBr prism). Liquid phase.³ Wavelength region 6–13 μ (NaCl prism). Nujol emulsion phase; 11–23 μ (KBr prism). KBr phase.

found. The indication obtained from this comparison about the structure of the cyclic polysulphide (I) is further strengthened, if Figs. 4, 5 and 6 on the one side is compared with Fig. 3 on the other. The much more intense and extensive vibrations of the thiolsulphinates are striking, and there can scarcely be any doubt about the significance, in any case as far as spectrochemical data are concerned. The exact wavelengths of the diethyl tri- and tetrasulphides are given in Table 1. Spectral correlation can thus be obtained from the cyclic system to the linear and has also been proved to exist within the series of the cyclic polysulphides in question. Thus, the probable S–S absorption of (I) is found at 20.75 μ (482 cm^{-1}), whereas a corresponding band occurs for (III) at 20.58 μ (486 cm^{-1}) and for (V) at 20.70 μ (483 cm^{-1}). The wavelengths for these three cyclic compounds are collected in Table 2. The agreement in the S–S specific absorption is excellent and probably reveals a strainless system, as the variations in the analogous frequency for the related 1,2-dithiolane

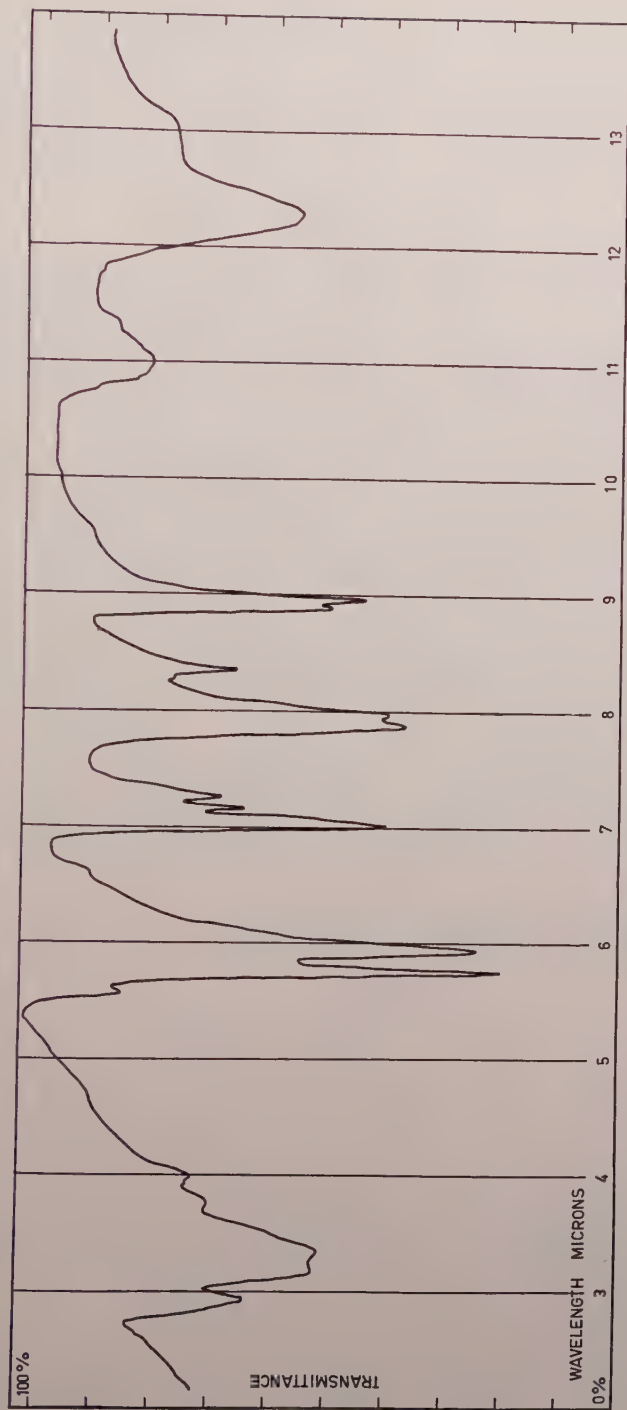


Fig. 7. IR absorption curve of tetrathio-di-glycolic acid (phase: solid pressed KBr plate) in the NaCl prism region.

derivatives were considerable (1). For the trisulphides normal values of the stretching frequencies are found, whereas the 1,2-dithiolanes showed unexpectedly high values (1). From other parts of the polysulphide spectra we may note that several bands occur in the region 11–14 μ . These are, however, surely connected with structural vibrations, as they mostly recur in the spectra of the desulphurization products although with changed mutual intensity. For that reason it is also clear that no assignments of the carbon–sulphur vibrations can be made.

From these spectrochemical investigations we conclude that the structures of the cyclic polysulphides are best characterized as (I b), (III b) and (V c). The author is aware of the fact that no absolutely decisive evidence has been presented but regards the work as strongly indicating unbranched sulphur chain structures. From the chemical properties given by BACKER and co-workers it can then be understood that the middle sulphur atom is easily removable. The course of analogous elimination reactions for polysulphides has successfully been studied with labelled atoms (5, 22). In the experimental part the substances are characterized with a nomenclature indicating unbranched structure.

Finally, in this investigation a study of a carboxylic substituted tetrasulphide, tetrathio-di-glycolic acid (XII), has also been made. The acid was first prepared by HOLMBERG (from thioglycolic acid and disulphur dichloride) (11) and was assigned a structure with an unbranched sulphur chain. The UV spectrum in alcoholic solution is shown in Fig. 2. A high intensity maximum occurs at 297 $m\mu$. The good agreement with the above mentioned spectra for diethyl tetrasulphide (13) and di-*n*-hexadecyl tetrasulphide (12) is noticeable. For the hexadecyl derivative an UV maximum is found at 300 $m\mu$. A high-intensity absorption at wavelengths longer than those observed for disulphides is to be expected here (see above), but it is interesting to note that the carboxylic groups seem to exert little or no influence on the spectral behaviour of the tetrasulphide group. For comparison attention may be called to the results for dithio-di-glycolic acid (XIII) (23) where no UV maximum could be detected in the region around 250 $m\mu$. The curve is reported again in Fig. 2. The inductive effect of the carboxylic groups here causes a hypsochromic shift to such an extent that a strong band in the vacuum ultraviolet region overlaps with a continuous absorption as a consequence. This interpretation is made possible by comparison with recent work on diselenides by BERGSON (24). Now assuming the tetrasulphide specific absorption as due to excitation of the middle S–S group electron pairs, we can understand that the carboxylic groups exert electronic influence only to a very small degree, as the inductive effect must then be transmitted through the C-attached sulphur atoms. The UV behaviour of this tetrasulphide substantiates well the spectrochemical knowledge about the S–S group and in fact presents strong evidence for the structure assumed by HOLMBERG.

The tetrasulphide acid showed also very interesting properties when examined in the infrared region. In Fig. 7 the spectrum is given for the sodium chloride region (solid phase), from which it appears that the carbonyl stretching frequency is split into two peaks at 5.75 μ (1739 cm^{-1}) and 5.94 μ (1684 cm^{-1}). This behaviour cannot be explained by the effect observed in certain diastereomeric acids (25), as *racemic* and *meso* dithiodilactic acid show single peaks at 5.82 μ (1718 cm^{-1}) and 5.80 μ (1724 cm^{-1}) (26). When examined in acetonitrile solution, on the other hand, a single top occurred at 5.75 μ (1739 cm^{-1}) indicating that the splitting in the solid state is due to some crystal effect. This is also likely from the fact that the bands related to the carbon–sulphur and sulphur–sulphur stretching vibrations also consist of

double peaks (see Table 1). The S-S frequencies are thus found at 20.25μ (494 cm^{-1}) and 21.08μ (474 cm^{-1}). The corresponding value for diethyl tetrasulphide is 20.85μ (480 cm^{-1}). It is suggested that the existence of two formally unequal carboxylic groups in the crystal lattice is due to an association through hydrogen bonds, e.g. such as (XIV), made possible by the long sulphur chain.

Experimental

A Beckman model DU quartz spectrophotometer was used for the *ultraviolet absorption determinations*. Different solvents, mostly alcohol, have been used, and the curves were recorded at least twice.

For the *infrared absorption measurements* a Perkin Elmer model 21 spectrophotometer was used. The substances were investigated as solid pressed potassium bromide plates (27), as Nujol emulsions or in liquid phase. The calibration is correct to $\pm 0.02 \mu$ for the sodium chloride region and to $\pm 0.04 \mu$ in that of potassium bromide.

The *molecular weight determinations* were performed by the ebullioscopic method with benzene and acetone as solvents (cf. 1).

*2,3,4,8,9,10-Hexathia-(5,5)-spiroundecane*¹ (Ib) was prepared according to BACKER and EVENHUIS (2) and recrystallized from benzene three times. M.p. 182° – 184° . Mol. wt. (benzene) Calc. 260.4; Found 245. The pentaerythrityl tetrabromide used was obtained as described in (28).

5,5-Dimethyl-1,2,3-trithiane (IIIb) was synthesized according to BACKER and TAMMA (3). The reaction mixture was fractionated *in vacuo* using a Vigreux column and gave a good yield of trisulphide. B.p. 114° – $116/12 \text{ mm}$. Mol. wt. (benzene) Calc. 166.3; Found 160.

*2,6,7,8-Tetrathia-(3,5)-spirononane*¹ (Vc). Preparation as described by BACKER and EVENHUIS (4). Three recrystallisations were made from light petroleum (b.p. 40° – 60°). M.p. 77° – 77.5° . Mol. wt. (acetone) Calc. 196.3; Found 187.

Diethyl disulphide was obtained in the usual manner by iodine oxidation of ethane thiol. It was purified by fractional distillation. B.p. $43^{\circ}/17 \text{ mm}$.

Ethyl ethanethiol-sulphinat was prepared according to the method (29) recommended by BACKER and KLOOSTERZIEL (15). Thus, diethyl disulphide (see above) was oxidized with perbenzoic acid in chloroform solution. B.p. 65° – $70^{\circ}/0.5\text{--}2 \text{ mm}$. The perbenzoic acid used was obtained as described in (30).

Diethyl trisulphide. Preparation from ethane thiol and thionyl chloride with ether as solvent according to HOLMBERG (11). B.p. $77^{\circ}/10 \text{ mm}$.

Diethyl tetrasulphide was obtained from sodium ethyl mercaptide and disulphur dichloride (cf. 31). Fractional, rapid distillation *in vacuo*. B.p. 93° – $94^{\circ}/7 \text{ mm}$. (CHAKRAVARTI (31) gave the coordinative formula, but he had accepted an incorrect structure for disulphur dichloride.)

Tetrathio-di-glycolic acid was synthesized from thioglycolic acid and disulphur dichloride in ether solution. The crude product was recrystallized once from water and five times from a mixture of ether and carbon disulphide. M.p. 111° – 112° . The tetrasulphide readily liberates two sulphur atoms to yield dithio-di-glycolic acid. Anal.

Subst., 58.29 mg; 6.88 ml of 0.0685N NaOH. Equ. wt. Calc. 123.1; Found 123.7.

¹ Numbering according to Ch. A.

Summary

The ultraviolet and infrared absorption spectra of some cyclic and linear polysulphides have been investigated. The results suggest that the BACKER trisulphides have a 1,2,3-trithiane structure.

The spectral behaviour of tetrathio-di-glycolic acid is discussed and compared to that of the related disulphide acid.

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Tryckt den 12 mars 1956

Uppsala 1956. Almqvist & Wiksells Boktryckeri AB

The preparation of α, α' -dimercapto-dicarboxylic acids

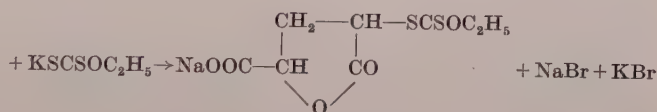
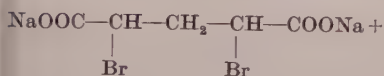
A survey of different methods

By LENNART SCHOTTE

With 3 figures in the text

Investigations in progress at this institute on sulphur derivatives related to glutaric and pimelic acid require the preparation of the α, α' -dimercapto acids. The accepted methods for introduction of a mercapto group into an organic molecule are in general applicable, but special attention must be paid to the stereochemical questions. The corresponding dibromo acids are starting materials.

In 1938 FREDGA (1) pointed out that mainly two methods are used for the synthesis of mercapto acids: the hydrolysis of the corresponding ethyl xanthates (2, 3) and the reduction of related disulphides. FREDGA investigated the adipic acid series (1) and found that the reactions could be performed without any steric conversions. Thus potassium ethyl xanthate and the diastereomers of α, α' -dibromoadipic acid gave stereochemically pure bis-ethylxanthogen acids, from which the mercaptans are obtained by hydrolysis with ammonia. Analogous results have recently been obtained for the pimelic acid derivatives by the present author (4, 5). If, on the other hand, the isomers of α, α' -dibromoglutaric acid are treated with potassium ethyl xanthate no bis-ethylxanthogen derivatives are isolated but rather a mono-xanthogen substituted γ -butyrolactone carboxylic acid. Preliminary experiments on resolution of the dibromo acids have fully illustrated the ease with which lactonization occurs.



The lactone carboxylic acid is obtained as a sodium salt dihydrate of rather low water solubility. Both the diastereomers of α, α' -dibromoglutaric acid give the same sodium salt, as has been established from infrared data and from a comparison of the related acids. As the yields are about the same in the two reactions and the salts separate during the treatment, an approach to the elucidation of the mechanism seems possible. The objection could of course be raised that the salt (and the related acid) might be a mixture of the diastereomers, but this does not seem very likely

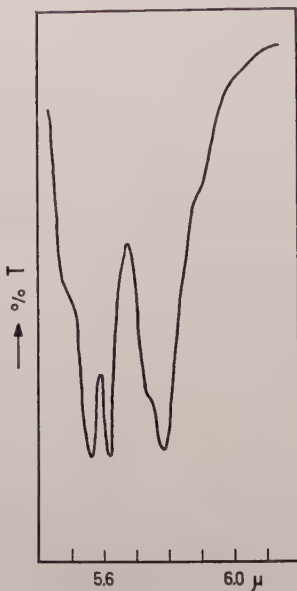


Fig. 1. IR absorption (5.4-6.2 μ) of α -xanthogen- γ -butyrolactone- γ -carboxylic acid. CaF_2 prism. KBr phase.

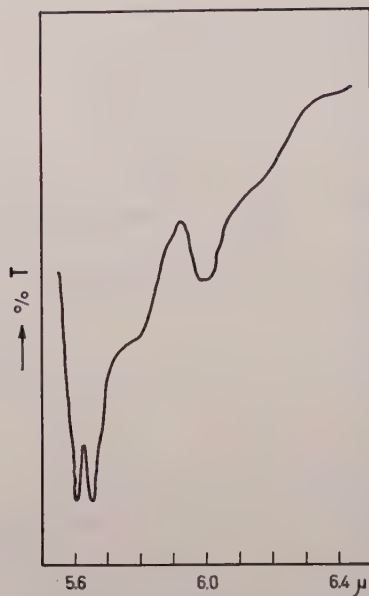


Fig. 2. IR absorption (5.5-6.5 μ) of acid potassium α -xanthogen- γ -butyrolactone- γ -carboxylate. CaF_2 prism. KBr phase.

from the spectral data. A steric conversion is, however, always possible to a certain extent, if the ring closure implies partly an S_N -mechanism. As the reaction conditions are analogous to those for the adipic and pimelic acid series it seems justified to associate the conversion with the lactone formation. In some cases carboxylic groups seem to favour S_N -mechanisms.

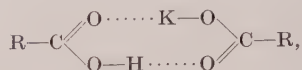
Analogous results have also been obtained when the free dibromoglutaric acids were treated with excess of potassium ethyl xanthate according to the descriptions given by BILLMANN (6) for the preparation of α -mercapto-*iso*-butyric acid with improved yields. An acid potassium salt of the above mentioned α -xanthogen- γ -butyrolactone- γ -carboxylic acid was isolated. Even here identical salts were obtained from different forms of the dibromo acids. The acid liberated from the acid potassium salt was identified with that obtained from the above sodium salt. Attempts to get derivatives of α,α' -dimercaptoglutaric acid from the lactones by ring opening as described in (7) for γ -butyrolactone as a model were unsuccessful. The reaction conditions in question do not allow any conclusions about stereochemical relationships to be drawn. On the other hand, no interactions occur e.g. with potassium thiolacetate under mild conditions. Moreover, reactions between potassium ethyl xanthate and dimethyl- α,α' -dibromoglutarates have not proved useful.

Before the discussion is extended to include other methods of preparation a closer study will be devoted to the infrared absorption spectra of the α -xanthogen- γ -butyrolactone- γ -carboxylic acid and the related salts. The substances have been investigated in the CaF_2 and NaCl prism region; the most interesting part of the spectra is that around 6 μ , where the frequencies assigned to the carbonyl stretching vibrations of carboxylic acids and lactones occur. Fig. 1 represents the region in



Fig. 3. IR absorption (5.5–6.5 μ) of sodium α -xanthogen- γ -butyrolactone- γ -carboxylate. CaF_2 prism. KBr phase.

question for the lactone acid as determined with a calcium fluoride prism. We note the peak at 5.79 μ , which is ascribed to the carbonyl stretching frequency of the carboxylic group. Moreover, the occurrence of a lactone $\text{C}=\text{O}$ group in the molecule is established by the double peak at 5.57 μ and 5.62 μ . γ -Lactones have been fairly extensively studied but are known to be characterized by single well-defined tops. The splitting into two peaks in this case is therefore noteworthy and is probably due to crystal effects as the substance in dioxane and N,N -dimethyl formamide shows a single top (at 5.58 μ). An analogous occurrence of double peaks for this vibration is also found in the case of the acid potassium and the sodium salt. In Fig. 2 it can be seen that these peaks occur for the acid potassium salt at 5.61 μ and 5.65 μ respectively. Another very interesting type of spectral behaviour is observed here, as no peaks can be found at the frequencies accepted for carboxylic groups and for salts of organic acids. Very extensive studies of the infrared spectra of salts have been made by LECOMTE and his co-workers (cf. 8), from which it can be concluded that they are characterized by bands between 1610 cm^{-1} and 1550 cm^{-1} (6.21 μ –6.45 μ). The only detectable characteristics in Fig. 2 which may be referred to vibrations in the carboxylic groups are the rather weak peak at 6.0 μ and possibly the shoulder at about 5.8 μ . This observation can probably be connected with the results of VITALE (9), who found that the molecular compounds $2\text{C}_6\text{H}_5\text{CH}_2\text{COOH} \cdot \text{C}_6\text{H}_5\text{CH}_2\text{COONa}$ and $2\text{CH}_3\text{COOH} \cdot \text{CH}_3\text{COONa}$ did not give any Raman absorption bands related to the carbonyl group. As a result of the spectral behaviour, structures of paraldehyde character (carbon oxygen bonds) were proposed. Such structures are impossible here, but it is suggested that an infrared absorption spectrum as in Fig. 2 may refer to a structure such as



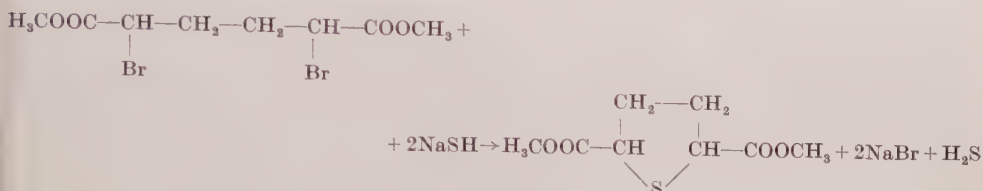
the properties of which must lie between those of acids and salts. A few experiments on linear acid salts seem to point in that direction. From Fig. 3, finally, it can be seen that the sodium salt of the lactone acid behaves normally from the infrared point of view with an absorption peak related to the carboxylic anion at 6.21μ . The lactone C=O group is as above characterized by double peaks, at 5.62μ and 5.65μ respectively. It should moreover be pointed out that the difference in frequency between the two lactone peaks for respective spectra decrease in the order acid, acid salt and normal salt.

As the substances investigated above contain the C=S linkage in the xanthogen group, it seems justified to discuss briefly the infrared behaviour of this bond although in fact it is beyond the scope of this paper. No adequate correlation has yet been established for the C=S linkage but the range $1400\text{--}1300 \text{ cm}^{-1}$ ($7.14\text{--}7.69 \mu$) has been suggested (cf. 10). The γ -butyrolactone derivatives mentioned above show absorption bands in the region $6.8\text{--}7.0 \mu$ ($1471\text{--}1429 \text{ cm}^{-1}$) which must be assigned to the stretching vibrations of the C=S linkage. Preliminary investigations of the diastereomers of α,α' -bis-ethylxanthogen pimelic acid, of ethylxanthogen acetic acid and α,α' -bis-ethylxanthogen acetone have substantiated such an interpretation for the C=S bond in the xanthogen groups.

The reduction of disulphide acids with zinc in ammoniacal solution certainly implies a convenient method for the preparation of mercapto acids but has in general found little application since the mercaptans are usually as readily available (by other methods) as the disulphides. For the adipic (1, 11) and pimelic (4) acid series the reactions between an alkali disulphide and α,α' -dibromo-dicarboxylic acids give not only disulphides but also sulphides. Moreover, the yields of disulphides are comparatively low. This seems partly due to the ring strain of the cyclic disulphides. In order to avoid the formation of linear polymers it has been found advisable to work with dilute solutions, which favours the ring closure to the monomeric derivatives. This is well illustrated for the diastereomers related to pimelic acid (5) and for *racem* 1,2-dithiolane-3,5-dicarboxylic acid (12, 13).

The direct introduction of the mercapto group into an organic molecule is often accomplished by the alkylation of alkali hydrosulphides. Alkyl sulphides are, however, by-products in these reactions, and with reactive halogenides the sulphide is quite predominant. Some α -halogen ketones react in that manner (cf. 14) and it has now been found that dibromo dicarboxylic acids and related esters also behave analogously. As the preceding results have indicated a pronounced tendency to lactonization for α,α' -dibromoglutaric acid it is perhaps better to start from related esters. Preliminary experiments showed, however, that a dimercapto acid was not obtained. The adipic acid series was then taken as a model for a closer investigation, as both the cyclic sulphide and disulphide diastereomers related to those of α,α' -dibromoadipic acid have been investigated by FREDGA (11, 1). Thus, *meso* α,α' -dibromoadipic acid in reaction with sodium hydrosulphide (aqueous solution) gave *cis(meso)*-thiophane-2,5-dicarboxylic acid and to a very small extent the expected dimercapto derivative. The ring closure with sulphide formation was observed even when excess of hydrosulphide was used. As far as the author could find the reaction proceeds without steric rearrangement. Ring closure to thiophane derivatives was also observed when the esters were used instead of the free acids. *Racem* and *meso* dimethyl α,α' -dibro-

moadipate treated in absolute methanol solutions with sodium hydrosulphide thus gave the corresponding sulphide esters.

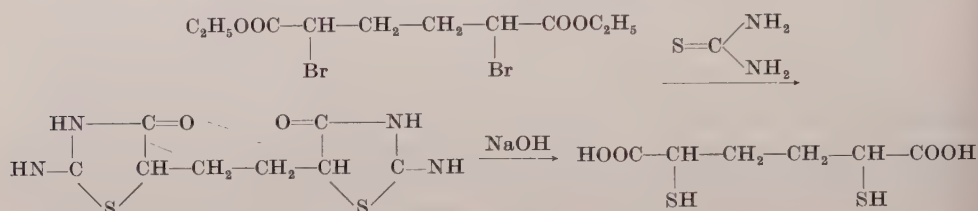


The sulphide esters were hydrolyzed with aqueous sodium hydroxide to yield the corresponding acids, probably slightly contaminated with the isomer in the crude products. A rearrangement was conclusively established in an analogous experiment with the diethyl ester. It seems justified to conclude from the above that the reactivity of these halogenides is great enough to cause further alkylation of the initially formed mercapto acids with ring closure as a consequence. The steric conditions for the adipic acid derivatives are especially favourable for ring closure. The racem $\rightleftharpoons$ meso rearrangements are likely to occur during the hydrolyses. This seems reasonable from the investigations of TURNER and HILL (15). From dimethyl α, α' -dibromoadipate and sodium sulphide they obtained *cis*(*meso*)-dimethyl thiophane-2,5-dicarboxylate, which on hydrolysis yielded an acid with an appreciably lower melting point than that given by FREDGA for the meso acid (11).

Another convenient method for the preparation of mercaptans is given in the hydrolysis of thiol esters. The thiol esters related to the dicarboxylic acids are easily prepared by the action of potassium thiol acetate on the bromo acids or esters. This method seems to be the best for the glutaric acid series and has been extensively investigated from the stereochemical point of view. α, α' -Dimercaptoglutaric acid can certainly be isolated in good yield, but the reaction proceeds with racem $\rightleftharpoons$ meso rearrangement. The details will be given later (13). Model investigations on the diastereomers of α, α' -dibromoadipic acid indicate that no racem $\rightleftharpoons$ meso conversion occurs in the reactions between the acids (in form of alkali salts) and the thiolacetate at room temperature. For the esters, on the other hand, rearrangements have been observed. It seems justified to connect this with the hydrolysis, as the presence of sulphur atoms in the α -positions to ester groups usually facilitates steric conversions. It should be pointed out that the adipic acid series is very suitable for investigations of the steric conditions in reactions involving the introduction of mercapto groups due to the solubility properties of the diastereomers of α, α' -dimercaptoadipic acid and related disulphides. The meso mercaptan has a low water solubility, much less than that of the diastereomer, but, on the other hand, the meso disulphide is about 30 times more soluble in water than the racemic isomer. A convenient way of determining the amounts of meso and racemic forms thus involves the preliminary isolation of the meso mercapto acid and subsequent iodine oxidation of the mother liquor to characterize any available racemic isomer.

The methods discussed above are undoubtedly the best for preparation of the α, α' -dimercapto acids in question. It has been regarded as adequate to exemplify the reactions on the well-known adipic acid series with supplementary experiments on α, α' -dibromoglutaric acid, where the lactonization tendency is predominant. It is, however, also of interest to investigate somewhat the reactions of the halogen acids with thiourea. The alkylation of thiourea by means of halides and subsequent alkaline

hydrolysis of the resulting isothiuronium salts to yield mercaptans in general involves a convenient method of preparation. It was certainly possible to prepare stereochemically pure *meso* α, α' -dimercaptoadipic acid in this way from the corresponding dibromo acid, but the yield was comparatively low. Moreover, the reaction conditions are too drastic to be satisfactory from the steric point of view. An interesting modification of this reaction is obtained, if an ester is used instead of the acid. Assuming an intramolecular condensation the reaction may proceed according to



This type of reaction was first investigated by OUCHI (16) for suberic acid. No molecular weight determination for the intermediate was given. In fact, a monomeric cyclic or polymeric linear intermediate will give the same mercaptan upon hydrolysis. In spite of the fact that attempts were made in the case of adipic acid to find conditions suitable for ring closure, solubility data of the product indicate a non-cyclic intermediate, which was therefore directly hydrolyzed to yield α, α' -dimercaptoadipic acid. As could be expected from the above ester treatments, $\text{racem} \rightleftharpoons \text{meso}$ rearrangement occurs during the hydrolysis.

The succinic acid derivatives have not been taken into consideration in the above discussion, as replacement reactions on α, α' -dibromosuccinic acid and related esters have proved unsuccessful. Special methods should be applied. α, α' -Dimercaptosuccinic acid can thus be obtained by addition of thiolacetic acid to acetylenedicarboxylic acid with subsequent hydrolysis of the acetyl groups (17).

Experimental

A. Reactions with potassium ethyl xanthate

α -Xanthogen- γ -butyrolactone- γ -carboxylic acid

(a) 7.25 g (0.025 mole) of *high-melting* α, α' -dibromoglutaric acid (m.p. 171°) was rapidly neutralized with an aqueous solution (30 ml) of 4.2 g (0.05 mole) of sodium bicarbonate. 9 g (0.056 mole) of potassium ethyl xanthate was added, and the mixture was kept at room temperature for three days with occasional shaking. A sodium salt began to separate rather soon. The yield of sodium α -xanthogen- γ -butyrolactone- γ -carboxylate was 4.8 g of crude product (62%). Recrystallizations from water gave 2.7 g of pure salt. The mother liquor was acidified with dilute sulphuric acid and placed in the refrigerator. The acid potassium salt of the xanthogen acid was isolated (1.0 g) and recrystallized twice from alcohol to yield 0.5 g with m.p. 158° – 159° (d.) (see below).

The acid was liberated from the sodium salt with dilute sulphuric acid and extracted with ether. Evaporation left a substance (in calculated yield) with m.p. 58° – 60° . The acid was recrystallized from benzene, m.p. 61° .

<i>Anal.</i> (of the sodium salt)		Na	H ₂ O
C ₈ H ₉ O ₅ S ₂ Na · 2 H ₂ O (308.30)	Calc.	7.46	11.69
	Found	7.53	11.8

Anal. (of the acid). Subst., 37.92 mg: 8.30 ml of 0.0734*N* NaOH. C₈H₁₀O₅S₂ (250.28). S Calc. 25.62; Found 25.44. — Subst., 115.18 mg: 6.72 ml of 0.0696*N* NaOH. Equ. wt. (phenolphthalein) Calc. 250.28; Found 246.3; — Subst., 124.37 mg: 7.05 ml 0.0696*N* NaOH. Equ. wt. (bromothymol blue) Calc. 250.28; Found 253.5.

The influence of the lactone group is clearly noticeable in the titrations.

(b) The same procedure as in (a) but with *low-melting* α,α' -dibromoglutaric acid (m.p. 131°). The yield of crude sodium salt was 5.2 g (67.5 %). Recrystallizations from water gave 2.8 g of salt. From the acidified mother liquor 1.0 g of acid potassium salt was isolated, which after recrystallization from alcohol showed m.p. 160° (d.) (0.4 g).

The acid liberated from the sodium salt as in (a) showed m.p. 58°–60° and recrystallized once from benzene, 61°–62°. The acids from (a) and (b) are identical (no melting point depression).

<i>Anal.</i> (of the sodium salt)		Na	H ₂ O
C ₈ H ₉ O ₅ S ₂ Na · 2 H ₂ O (308.30)	Calc.	7.46	11.69
	Found	7.22	11.5

(c) 14.5 g (0.05 mole) of *high-melting* α,α' -dibromoglutaric acid (m.p. 171°) was added to a 50 ml aqueous solution of 32 g (0.2 mole) of potassium ethyl xanthate. The mixture was kept at room temperature for three days. The oil which separated (mostly carbon disulphide and xanthogenic acid) was removed with ether and the remaining solution acidified with dilute sulphuric acid. An oil precipitated which soon crystallized upon cooling. 5.4 g (40 %) of crude acid potassium salt of α -xanthogen- γ -butyrolactone- γ -carboxylic acid was thus obtained. M.p. about 152° (d.). The salt was recrystallized twice from alcohol to give 2.9 g with m.p. 161°–162° (d.). The melting point depends somewhat on the rate of heating.

The acid liberated from this salt is identical with that prepared in (a) and (b). M.p. 60°–61°.

<i>Anal.</i> (of the salt)		K	S
C ₈ H ₉ O ₅ S ₂ K + C ₈ H ₁₀ O ₅ S ₂ (538.65)	Calc.	7.26	23.81
	Found	7.34	24.09

(d) The same procedure as in (c) except that *low-melting* α,α' -dibromoglutaric acid (m.p. 134°) was used. From 0.025 mole of this acid 4.7 g (70 %) of salt was obtained. M.p. about 150° (d.). Recrystallizations (twice) from alcohol gave 1.9 g of pure acid potassium salt with m.p. 160° (d.).

The infrared spectrum of this salt is identical with that of the salt obtained in (c).

(e) If the sodium bicarbonate in procedure (a) is changed for potassium bicarbonate, the acid potassium salt is obtained after acidification. 4 g (59 %) of crude salt with m.p. about 155° (d.) was isolated. Recrystallization from alcohol yielded 1.6 g of the pure substance. M.p. 161° (d.). The salt is identical with those obtained above.

B. Reactions with alkali hydrosulphide

The diastereomers of thiophane-2,5-dicarboxylic acid

(a) 9.1 g (0.03 mole) of *meso* α,α' -dibromoadipic acid (18) was neutralized in the cold with 5.04 g (0.06 mole) of NaHCO_3 , dissolved in 75 ml of water. An aqueous solution (30 ml) of sodium hydrosulphide (prepared from NaOH in water by saturating with hydrogen sulphide) was added. The mixture was left at room temperature for two days and then acidified with dilute sulphuric acid. Extraction with ether eventually gave 4.5 g (85 %) of a substance with m.p. $130^\circ\text{--}140^\circ$. Recrystallizations from water yielded 3.0 g of pure *meso* thiophane-2,5-dicarboxylic acid, m.p. $144^\circ\text{--}146^\circ$. The presence of a small amount (about 0.1 g) of α,α' -dimercaptoadipic acid was established by iodine oxidation and by the preparation of the dibenzyl derivative.

Anal. Subst. (m.p. $130^\circ\text{--}140^\circ$), 52.12 mg; 8.23 ml of 0.0690N NaOH . Equ. wt. Calc. 88.06; Found 91.8.

Subst. (m.p. $144^\circ\text{--}146^\circ$), 50.33 mg; 8.20 ml of 0.0690N NaOH . Equ. wt. Calc. 88.06; Found 89.0.

(b) Analogous procedure to that in (a) except that excess of sodium hydrosulphide was used (0.15 mole of NaSH ; total volume 125 ml). 4.7 g (89 %) of crude product was obtained with m.p. $130^\circ\text{--}140^\circ$. Purification from water gave the pure *meso* sulphide, m.p. $144^\circ\text{--}146^\circ$.

Anal. Subst., 51.37 mg; 8.38 ml of 0.0690N NaOH . Equ. wt. Calc. 88.06; Found 88.8.

(c) 33.2 g (0.10 mole) of *meso* dimethyl α,α' -dibromoadipate (prepared from the acid with diazomethane (cf. 19)) was dissolved in 300 ml of absolute methanol. This solution was added dropwise with cooling and stirring to 200 ml of absolute methanol which contained 0.22 mole of NaSH . The stirring was continued for one hour and the mixture left over-night. Most of the methanol was distilled off and water added to dissolve the salts. The separated ester was hydrolyzed with 25 % aqueous NaOH at reflux temperature (20 minutes). After acidification with sulphuric acid the acid was removed with ether. Evaporation of the solvent left a substance with m.p. $127^\circ\text{--}140^\circ$. Repeated recrystallizations from water gave 6.2 g (35 %) of pure *meso* thiophane-2,5-dicarboxylic acid, m.p. 143° .

Anal. Subst., 62.10 mg; 10.17 ml of 0.0690N NaOH . Equ. wt. Calc. 88.06; Found 88.5.

(d) The procedure described in (c) was repeated, in half scale, with *racem* dimethyl α,α' -dibromoadipate (obtained from the acid and diazomethane). The acid finally obtained was recrystallized twice from water to yield pure *racem* thiophane-2,5-dicarboxylic acid (3.5 g; 40 %). The melting point was $164^\circ\text{--}165^\circ$.

Anal. Subst., 59.80 mg; 9.80 ml of 0.0690N NaOH . Equ. wt. Calc. 88.06; Found 88.4.

(e) 9 g (0.025 mole) of *meso* diethyl α,α' -dibromoadipate (m.p. 66° (20)) was treated with an ethanolic solution of KSH . Total volume: 225 ml. The reaction was performed as in (c). 3.4 g (77 %) of crude sulphide was obtained after hydrolysis, m.p. $125^\circ\text{--}135^\circ$. Crystallization from water gave a first acid fraction with m.p. $158^\circ\text{--}161^\circ$. Another crystallization of this acid from water yielded pure *racem* thiophane-2,5-dicarboxylic acid (m.p. $165^\circ\text{--}166^\circ$).

Anal. Subst., 44.47 mg; 7.30 ml of 0.0690N NaOH . Equ. wt. Calc. 88.06; Found 88.3.

The second fraction in the above crystallization showed the melting point 128°–135°. No complete separation of the diastereomers is possible from this mixture.

Anal. Subst. (m.p. 128°–135°), 46.10 mg; 7.55 ml of 0.0690*N* NaOH. Equ. wt. Calc. 88.06; Found 88.5.

All the pure acids isolated have been identified with authentic samples.

C. Reactions with potassium thiolacetate

The diastereomers of α,α' -dimercaptoadipic acid

(a) 9.1 g (0.03 mole) of *meso* α,α' -dibromoadipic acid was neutralized with KHCO_3 in 100 ml of water. A solution of 0.08 mole of potassium thiolacetate (prepared from 6.1 g (0.08 mole) of redistilled thiolacetic acid and 4.5 g (0.08 mole) of KOH in water) in 30 ml of water was added and the mixture allowed to stand at room temperature for two days. After acidification the acetylated compound was extracted with ether and after removal of the solvent hydrolyzed, simply by dissolving in excess of aqueous NaOH. Next day the solution was acidified. *Meso* α,α' -dimercaptoadipic acid separated and was recrystallized from water. 3.7 g (59%) with m.p. 186° (d.) was obtained. No racemic isomer could be found in the mother liquors.

Anal. Subst., 48.05 mg; 6.35 ml of 0.0721*N* NaOH. Equ. wt. Calc. 105.1; Found 105.0. — Subst., 42.10 mg; 4.02 ml of 0.0999*N* iodine. Equ. wt. Calc. 105.1; Found 104.8.

(b) 9.1 g (0.03 mole) of *meso* α,α' -dibromoadipic acid was esterified with diazomethane and the ester dissolved in 100 ml of absolute methanol. A methanol solution (100 ml) of potassium thiolacetate was prepared from 5.6 g (0.074 mole) of thiolacetic acid and an equivalent amount of KOH (4.1 g) in methanol and added to the ester solution. The separation of KBr began at once. Next day most of the methanol was removed *in vacuo* and the residue poured into water. The acetylated mercapto ester was extracted with ether, which after evaporation left a solid. This substance was directly hydrolyzed with KOH in water (11 g of KOH in 100 ml of water at 60°–70° during 3.5 hours). Upon acidification *meso* α,α' -dimercaptoadipic acid separated and after recrystallization from water showed m.p. 184° (d.). The yield was 3.6 g (57%).

Anal. Subst., 59.80 mg; 7.89 ml of 0.0721*N* NaOH. Equ. wt. Calc. 105.1; Found 105.1. — Subst., 32.03 mg; 3.09 ml of 0.0999*N* iodine. Equ. wt. Calc. 105.1; Found 103.8.

From the mother liquors 1.3 g (21%) of *racem* 1,2-dithiane-3,6-dicarboxylic acid could be isolated after oxidation with iodine. M.p. 261° (d.).

Anal. Subst., 49.10 mg; 6.54 ml of 0.0721*N* NaOH. Equ. wt. Calc. 104.1; Found 104.1.

(c) The reaction described in (b) was repeated with *racem* α,α' -dibromoadipic acid (18) instead of the *meso* isomer. 9.1 g (0.03 mole) of acid was used and the total methanol volume was 80 ml. The hydrolysis of the ester was performed with 11 g of KOH in 60 ml of water at 70°–80° during 4 hours. From the acidified solution 2.2 g (35%) of *meso* α,α' -dimercaptoadipic acid, m.p. 185° (d.), was obtained (one recrystallization from water).

Anal. Subst., 64.63 mg; 8.49 ml of 0.0721*N* NaOH. Equ. wt. Calc. 105.1; Found 105.6. — Subst., 47.78 mg; 4.62 ml of 0.0999*N* iodine. Equ. wt. Calc. 105.1; Found 103.5.

The mother liquors were oxidized with iodine to yield the racemic disulphide component. 2.5 g (40 %) of *racem* 1,2-dithiane-3,6-dicarboxylic acid was isolated (one recrystallization from acetone-water) with the m.p. 260° (d.).

Anal. Subst., 59.15 mg; 7.91 ml of 0.0721N NaOH. Equ. wt. Calc. 104.1; Found 103.7.

D. Reactions with thiourea

The diastereomers of α,α' -dimercaptoadipic acid

(a) 10 g (0.033 mole) of *meso* α,α' -dibromoadipic acid was treated with excess (8 g) of thiourea in 50 ml of water after rapid neutralization with NaHCO_3 (5.5 g) in 30 ml of H_2O . Refluxing was continued for four hours. 8 g of NaOH in 30 ml of water was added and the *isothiuronium* salt formed hydrolyzed at reflux temperature for six hours. After acidification with dilute sulphuric acid *meso* α,α' -dimercaptoadipic acid was isolated. The yield was 2.2 g (32 %) and the melting point after two recrystallizations 184° – 185° (d.). It could be established that no rearrangement to the racemic isomer occurred during the reaction. Moreover, it has been found that the comparatively long reaction times are necessary.

Anal. Subst., 52.40 mg; 7.18 ml of 0.0690N NaOH. Equ. wt. Calc. 105.1; Found 105.8. — Subst., 52.01 mg; 5.00 ml of 0.0999N iodine. Equ. wt. 105.1; Found 104.1.

(b) 18 g (0.05 mole) of *meso* diethyl α,α' -dibromoadipate was dissolved in 200 ml of ethanol and added dropwise with stirring to a solution of 8.0 g (0.11 mole) of thiourea in 300 ml of ethanol. The addition time was 1.5 hour. The mixture was then gently refluxed for two hours. A faint yellow substance separated on cooling (evaporation of the solvent yielded a further quantity) and was dissolved for hydrolysis in an aqueous solution (180 ml) of 12 g NaOH. The hydrolysis required 2.5 hours and was performed in nitrogen atmosphere. From the acidified solution 5 g (47.5 %) of α,α' -dimercaptoadipic acid (mixture of the diastereomers) deposited (m.p. 155° – 170°). After three recrystallizations from water 3.4 g pure *meso* acid was obtained, m.p. 186° (d.).

Anal. Subst., 64.83 mg; 8.86 ml of 0.0696N NaOH. Equ. wt. Calc. 105.1; Found 105.1. — Subst., 43.50 mg; 4.19 ml of 0.0999N iodine. Equ. wt. Calc. 105.1; Found 103.9.

Oxidation of the mother liquors with iodine gave 1 g of *racem* 1,2-dithiane-3,6-dicarboxylic acid. M.p. 265° (d.).

Anal. Subst., 57.49 mg; 7.95 ml of 0.0690N NaOH. Equ. wt. Calc. 104.1; Found 104.8.

In C and D all titrations of the mercapto acids with NaOH have been performed with bromothymol blue as an indicator.

The infrared absorption measurements

A Perkin Elmer model 21 spectrophotometer equipped with NaCl and CaF_2 prisms was used for the determinations. The substances were examined as solid pressed potassium bromide plates and as Nujol mulls. The calibration of the absorption curves is correct to $\pm 0.01 \mu$.

Summary

Different methods for the preparation of α, α' -dimercapto-dicarboxylic acids have been discussed with special reference to stereochemical points of view.

The adipic acid series was taken as a model and the lactonization tendency for the glutaric acid derivatives has been pointed out.

Racem $\rightleftharpoons$ meso rearrangements have been established in case of the ester reactions.

The infrared absorption behaviour of the carbonyl groups in acid salts and the C—S linkage in xanthogen compounds have been briefly discussed.

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Quasi-racemic compounds between optically active positional isomers of methyl-substituted long-chain carboxylic acids

By Bo HALLGREN

With 4 figures in the text

Racemic 9-methyloctadecanoic acid was first synthesized by STÄLLBERG-STENHAGEN in connection with synthetic work on tuberculostearic acid. She reports a melting point of 38.7–39.2°. This acid has since been prepared by several synthetic routes by a number of other authors (see Table 1). There is general agreement about the high melting point of this acid compared with 8- and 10-methyl isomers. No polymorphism has been observed. It appeared of interest to study the properties of the optically active forms of the 9-methyl-substituted acid particularly in view of the fact that racemic 9-methyloctadecanoic acid has recently been found to possess a very interesting crystal structure (11).

In the present work the racemic acid and the dextrorotatory 9D-methyloctadecanoic acid were prepared from DL- and D-methyl hydrogen β -methylglutarate by two successive chain-lengthenings using the Kolbe electrolysis (cf. 10, 12). Mixed electrolyses with *n*-nonanoic acid performed as described in the experimental part gave via the methyl esters 3DL-methyldodecanoic acid, b.p. 115°, 0.20 mm, and (+)-3D-methyldodecanoic acid, b.p. 135–137°, 0.5 mm, $[\alpha]_D^{24.5} + 4.78^\circ$ respectively. The yield was about 30 per cent of the theoretically possible, calculated on the half-ester used. In the second chain-lengthening step each of the two free methyldodecanoic acids were subjected to mixed electrolysis with methyl hydrogen suberate. This gave in yields of about 25 per cent 9DL-methyloctadecanoic acid and (+)-9D-methyloctadecanoic acid respectively. After crystallization from low-boiling light petroleum the melting point of the racemic acid was 38.7–39.2°, identical with the figure given by STÄLLBERG-STENHAGEN (see Table 1) for material synthesized along a quite different route. The melting point of the amide now prepared agreed closely with that given by STÄLLBERG-STENHAGEN (see Table 1). The optically active acid after crystallization from methanol at a temperature of -15° melted at 13.3–13.4°. The m.p. of the amide was 65.5–65.9°. However, material obtained by hydrolysis of the amide after crystallization from methanol at -15° melted at 16.4–16.7°. After solidification the acid remelted at 12.5–12.9°. The dimorphism of the optically active acid was confirmed by a study of the X-ray powder diagrams.

The liquidus curve for the binary system of the antipodes of 9-methyloctadecanoic

Table 1.

	M.p. acid °C	M.p. amide °C
9DL-Methyloctadecanoic acid	38.7-39.2 present study 38.7-39.2 (1) 38.5-39.1 (2) 38.5-39 (3) 40 -41 (4) 37.5-38.5 (5)	69.9-70.1 and 61.3-61.5 present study 70.0-70.5 and 61.3-61.5 (1) 69.8-70.5 and 62.8 (2) 69.5-70 (3) 70 (4)
(+)-9D-Methyloctadecanoic acid	12.5-12.9 and 16.4-16.7 present study	33.5 tr., 65.5-65.9 present study
10DL-Methyloctadecanoic acid	20 -21 (6) 25.4-26.1 (7) 25.8-26.1 and 20.9-21.6 (1) 23.5-25.8 (8) 25.5-26 (3) 25.8-26.4 and 20.9-21.2 (9)	76 -77 (6) 77.5-79.2 (7) 79.3 (1) 77 -78 (8)
(+)-10L-Methyloctadecanoic acid	13 -13.5 (7) 12.5-12.8 (1) 12.3-12.8 (10)	75.5-76.4 (7) 76.2-76.6 (1)
(-)-10D-Methyloctadecanoic acid	12.8-13.4 (7) 12.4-12.8 (1) 12.5-12.9 (10)	75.1-76.3 (7) 76.4-76.6 (1) 75.7-76.2 (10)

acid is shown in Fig. 1. It is seen that the racemic compound dominates the diagram. The corresponding diagram for the antipodes of 10-methyloctadecanoic acid taken from the paper of STÄLLBERG-STENHAGEN is also reproduced in Fig. 1. Two crystal modifications were observed for the racemic acid in this case and the liquidus curve of Fig. 1 refers to the higher-melting form. In view of the strong tendency towards formation of racemic compounds especially in the case of the 9-methyl acid it appeared of interest to study binary systems containing one antipode of 9- and one of 10-methyloctadecanoic acid in order to see whether quasi-racemic compounds (13) are formed. The results are given in Figs. 2 and 3. 9D- and 10L-Methyloctadecanoic acid form a quasi-racemic compound melting at 18.6-18.7° whereas 9D- and 10D-methyloctadecanoic acid form an eutectic, melting at 1.9-2.5°.

In order to obtain the curve shown in Fig. 2 it is necessary to keep the mixtures in the solid state for a short time. If the melting point is taken immediately after rapid solidification of the mixture lower and very diffuse melting points were obtained. This indicates the existence of a lower-melting modification but the corresponding melting point curve could not be constructed. Mixtures of (+)-9D- and (-)-10D-methyloctadecanoic acid on very rapid cooling solidified in glassy form. On slow heating, mixtures containing 20 per cent or less of one component turned crystalline but between 20 and 48 per cent the glassy solid liquifies on heating. When kept at -15° overnight specimens in the 20-48 per cent region crystallize but on heating melt over a broad range of temperature, evidently because of separation of the components. The 50 per cent mixture usually melted between 1.9-2.5°. Sometimes

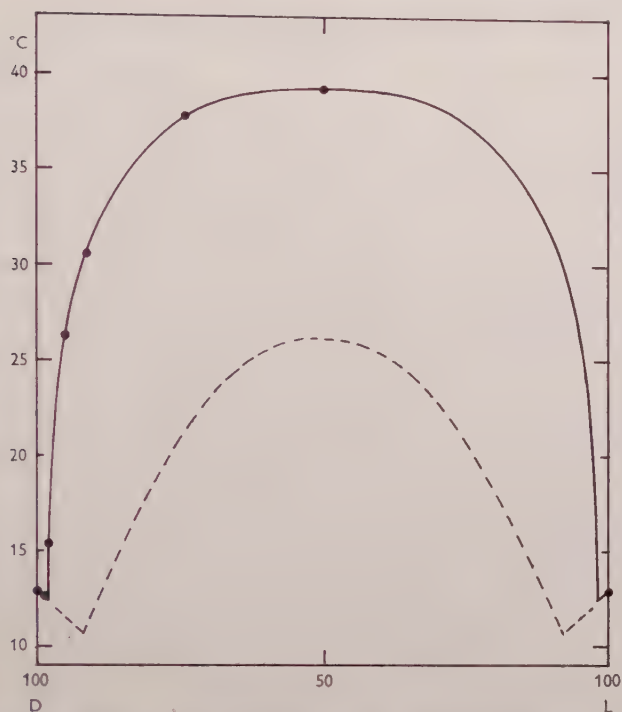


Fig. 1. Melting point diagram (liquidus curve) of the binary system of the enantiomorphs of 9-methyloctadecanoic acid. The corresponding curve for the enantiomorphs of 10-methyloctadecanoic acid taken from the paper of STÄLLBERG-STENHAGEN (1) is included (dashed line) for comparison.

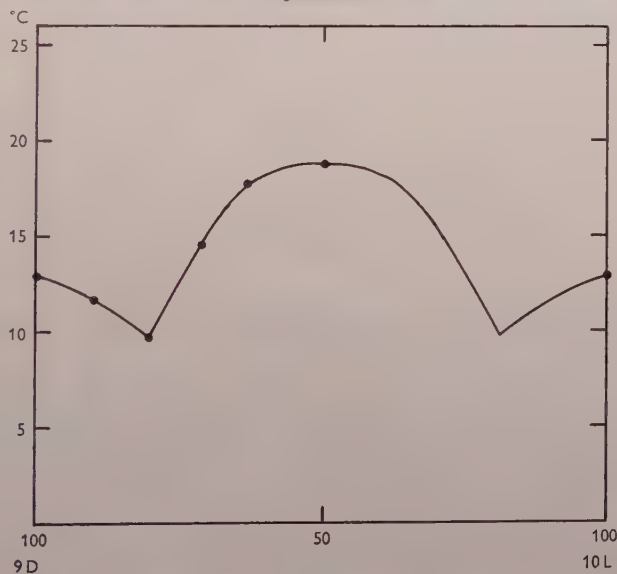


Fig. 2. Melting point diagram (liquidus curve) of the binary system composed of (+)-9D-methyloctadecanoic acid (low-melting form) and (+)-10L-methyloctadecanoic acid.

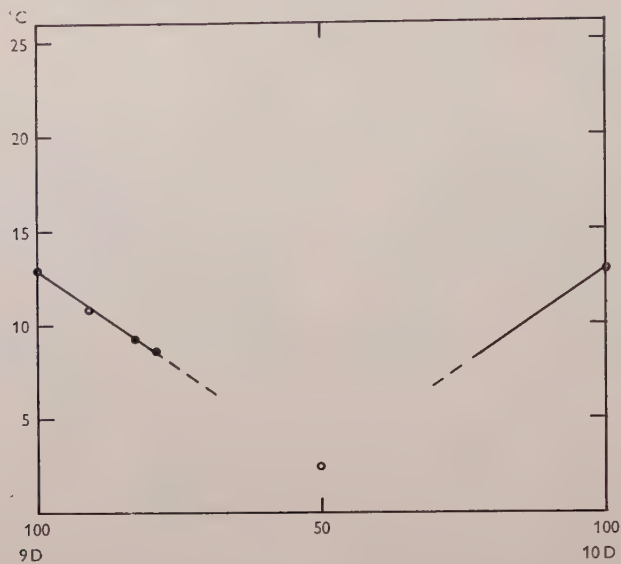


Fig. 3. Melting point diagram of the binary system composed of (+)-9D-methyloctadecanoic acid and (-)-10D-methyloctadecanoic acid.

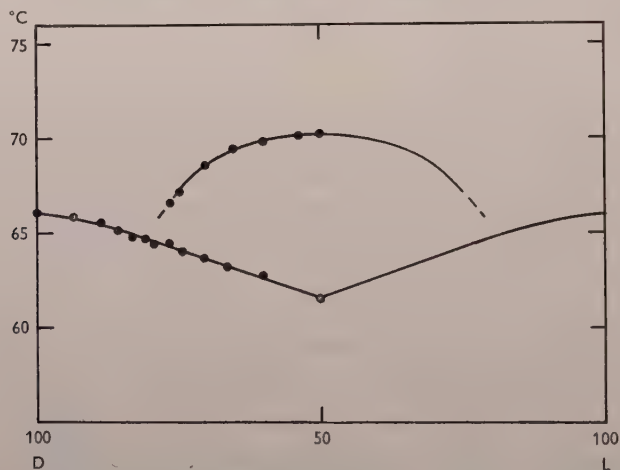


Fig. 4. Melting point diagram of the binary system composed of the enantiomorphs of 9-methyloctadecanamide.

however, the melt remained translucent until the temperature had been raised to about 3.5° . The binary system of enantiomorphous amides is shown in Fig. 4. The figure shows that the higher melting form of the amide is a racemic compound, whereas the low-melting form, which is obtained by solidification of the melt, is a simple eutectic. The thermal behaviour of the enantiomorphs do not indicate more than one crystalline form, but the diffraction pattern-temperature diagram (16) shows that specimens crystallized from ether on heating undergo a transition at 33.5° .

Experimental¹**(+)-3D-Methyldodecanoic acid**

Sodium (0.8 g) was dissolved in methanol (300 ml, LKB-Produkter "pro analysi") and nonanoic acid (9.9 g, 0.0626 mole; the commercial product was distilled through an electrically heated and packed column, b.p. 105°, 0.8 mm. n_D^{25} 1.4305) and (+)-methyl 3D-methyl-4-carboxybutanoate (10 g, $\alpha_D^{22.0} - 0.64^\circ$ (homogeneous; *l*, 1)) added. The electrolysis was carried out between platinum electrodes, using a current of 7–10 amps. Excessive foaming was avoided by cooling the outside of the electrolysis vessel with tap water. The electrolysis was continued until the pH of the solution had risen to 7. This happened after about 40 minutes. Additional amounts of 9.9 g of nonanoic acid and 10 g of the active half-ester were added, and the electrolysis continued until the pH again reached 7. This was repeated three times so that in all 49.5 g (0.313 mole) of nonanoic acid and 50 g (0.313 mole) of the active half-ester were electrolysed. Methanol had to be added from time to time to replace loss due to evaporation. The reaction mixture was poured into water, and the mixture extracted with five portions of ether. The combined ether extract was washed with sodium carbonate solution and with water. The ether solution was dried with sodium sulphate, the solvent removed, and the residue distilled. The fractions boiling between 86–88°, 0.25–0.30 mm, were collected (49.2 g) and subjected to hydrolysis by means of potassium hydroxide (35 g), water (70 ml), and ethanol (200 ml of 99.5%). The mixture was refluxed for 3 hours. In order to remove neutral products (*n*-hexadecane) the mixture was diluted with water and extracted three times with ether. The alkaline water-ethanol solution was acidified by means of hydrochloric acid and extracted with ether. The ether solution was dried and the solvent distilled off. In order to remove most of the dibasic acid the residue (35.2 g) was dissolved in 200 ml of light petroleum (b.p. 20–40°) and kept at a temperature of –15° overnight. The white microcrystalline material that had separated was filtered off. The light petroleum was evaporated and the residue distilled, giving 18.4 g of (+)-3D-methyldodecanoic acid, b.p. 135–137°, 0.5 mm. n_D^{25} 1.4405. $d_4^{24.5}$ 0.8848.

Optical rotation: $\alpha_D^{24.5} + 4.23^\circ$ (homogeneous; *l*, 1)
 $[\alpha]_D^{24.5} + 4.78^\circ$ $[M]_D^{24.5} + 10.2^\circ$

This acid has previously been prepared in this laboratory by R. CAVANNA (15), who found an optical rotation of $\alpha_D^{22} + 4.16^\circ$ (homogeneous; *l*, 1).

(+)-9D-Methyloctadecanoic acid

0.4 g of sodium was dissolved in 250 ml of methanol, and methyl hydrogen suberate (15.45 g, 0.082 mole; prepared from suberic acid (m.p. 140°) and methyl suberate by the method described in Org. Synth. Coll. Vol. II, p. 276) and (+)-3D-methyldodecanoic acid (17.60 g, 0.082 mole) added. The mixture was electrolysed for 45 minutes, a current of 4–9.5 amps. being used. About 300 ml of water was added and the mixture extracted with five portions of ether. The ether solution was washed with sodium carbonate solution and with water, dried, and the ether distilled off.

¹ Crystal spacings, optical rotations and melting point data using capillary tubes were determined as described by SERCK-HANSEN, STÄLLBERG-STENHAGEN and STENHAGEN (14). These authors also discuss the stereochemical nomenclature.

The residue (about 23 g) was subjected to hydrolysis, and the free acids taken up in ether in the usual manner (see above). The residue obtained after removal of the ether was dissolved in about 200 ml of light petroleum (20–40°) and kept at room temperature for about 3 hours. The white, microcrystalline material that separated (dibasic acid) was filtered off. The light petroleum was distilled off and the residue fractionated by distillation. (+)-9D-Methyloctadecanoic acid distilled over at a temperature of 140–179°, the pressure being 0.6–0.8 mm. For purification the active methyloctadecanoic acid was dissolved in light petroleum (20–40°) and kept at –20° for several hours. Small amounts of white, microcrystalline material separated and was filtered off. This procedure was repeated three times until no more material separated from light petroleum at –20°. The light petroleum was removed and a residue of 4.47 g of pure 9D-methyloctadecanoic acid obtained. After crystallization from methanol, the melting point was 13.3–13.4°. Acid obtained by hydrolysis of the amide described below, melted, after crystallization from methanol, at 16.4–16.7°. After solidification remelting occurred at 12.5–12.9°. Some days later the m.p. had risen to 12.6–13.2°, evidently by partial conversion to the high-melting form. It seems likely that similar phenomena are responsible for the slightly divergent melting points reported for the active 10-methyloctadecanoic acids (1, 7). The X-ray diffraction pattern of the high-melting form was identical with that of the DL-acid. In this case the crystal structure does not give rise to the normal series of 00*l* reflections (11). The low-melting form showed a long spacing of 44.7 Å. n_D^{25} 1.4511. d_4^{24} 0.8733.

Optical rotation: $\alpha_D^{21} + 0.04^\circ \pm 0.01^\circ$ (homogeneous; *l*, 1)
 $[\alpha]_D^{21} + 0.05^\circ$ $[M]_D^{21} + 1.5^\circ$

Anal.: Calc. for C₁₉H₃₈O₂ (298.5) C, 76.45; H, 12.83 %
 Found C, 76.10; H, 12.81 %

(+)-9D-Methyloctadecanamide

0.5 g of the acid was converted into the acid chloride by means of oxalyl chloride (0.35 ml) in benzene solution (4 ml). The mixture was kept at a temperature of 30–35° for 4 hours. Excess oxalyl chloride was distilled off at a temperature of 60° under reduced pressure. The residue was cooled to room temperature and added drop by drop into 20 ml of cold (+4°) aqueous ammonia. The mixture was shaken vigorously for 5 minutes. The mixture was poured into a wide vessel, water added, and allowed to stand overnight. The amide was extracted by means of four portions of ether. The ether solution was washed with water, dried, and the ether distilled off. After three crystallizations from ether the amide formed small, lustrous plates of m.p. 65.5–65.9°. The X-ray diffraction pattern gave a long spacing of 36.5 Å at room temperature. A diffraction pattern-temperature diagram taken with a recording camera (16) showed a sharp transition at 33.5° to a form with a longer long spacing which persisted up to the melting point. Material solidified from the melt also possessed this crystal form, which has a long spacing of 38.8 Å at room temperature.

Optical rotation: $\alpha_D^{24} + 0.03^\circ \pm 0.01^\circ$ (chloroform; *l*, 1; *c*, 43)
 $[\alpha]_D^{24} + 0.07^\circ$ $[M]_D^{24} + 2.1^\circ$

Anal.: Calc. for C₁₈H₃₇ON (297.5) N, 4.71 %
 Found N, 4.66 %

9DL-Methyloctadecanoic acid and the corresponding amide were prepared by an analogous series of reactions, using 3DL-methyldodecanoic acid as starting material. Thermal data are given in Table 1. The diffraction pattern of the higher melting form of the amide did not show the usual series of 00 *l* reflections. The low melting form shows a diffraction pattern identical with that obtained for the enanthiomorph after solidification of the melt.

(-)-10L,13D-Dimethyldocosane

Distillation of the non-saponifiable fraction gave (-)-10L,13D-dimethyldocosane (2.45 g) b.p. 156°, 0.3 mm. $n_D^{25.0}$ 1.4459. d_4^{25} 0.7971.

Optical rotation: $\alpha_D^{25.8} - 0.78^\circ$ (homogeneous; *l*, 1)
 $[\alpha]_D^{25.8} - 0.98^\circ$ $[M]_D^{25.8} - 33.2^\circ$

Anal.: Calc. for C₂₄H₅₀ (338.6) C, 85.12; H, 14.88 %
 Found C, 85.10; H, 14.84 %

Technique used in the study of binary systems

The different mixtures were prepared by weighing into small test tubes the amounts of the components sufficient to give at least 20 mg of mixture. The mixture was dissolved in a small amount of ether. The solvent was allowed to evaporate at room temperature. The last traces of solvent were removed *in vacuo*. The solid mixtures of the amides were transferred to capillary tubes for melting point determinations in the usual way, using a bath of liquid paraffin. The mixtures of the acids were left in the small test tubes and after solidification in a suitable manner the melting points were determined in a water bath.

Summary

The synthesis of 9DL-methyloctadecanoic acid and (+)-9D-methyloctadecanoic acid by two successive chain-lengthenings of racemic and (+)-methyl hydrogen β -methylglutarate respectively is described. A study of binary systems formed between enanthiomorphs of 9- and 10-methyloctadecanoic acid has shown that 9-methyloctadecanoic acid has a very strong tendency to form a racemic compound and that the enanthiomorphs of this acid form quasi-racemic compounds with the configurationally different enanthiomorphs of 10-methyloctadecanoic acid. Binary systems between configurationally similar enanthiomorphs of these two acids show only a simple low-melting eutectic.

This study is a part of investigations of the chemistry of higher branched chain fatty acids carried out in this institute. It has been financially aided by grants from *Statens Medicinska Forskningsråd* to S. and E. STENHAGEN.

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Stereochemical studies on dicarboxylic acids

IV. An attempt to indicate mesoid or racemoid configuration by infrared absorption

By LENNART SCHOTTE

With 4 figures in the text

In a number of papers the infrared absorption curves of some substituted dicarboxylic acids have been considered (1, 2, 3, 4). The infrared approach to stereochemical problems has proved valuable and has hitherto been applied mostly to the study of diastereomers with two similar asymmetric carbon atoms. Especially in the succinic acid series significant effects of different spatial distribution on the infrared absorption is observed. The splitting of the carbonyl stretching frequency for the racemic isomer into double peaks in such cases has been regarded as the most outstanding difference in the spectra of a pair of diastereomers.

The infrared work has now been extended to include the investigation of the high- and low-melting forms of α -ethyl- α' -methyl-succinic acid and α -ethyl- α' -methyl-glutaric acid in the sodium chloride prism region. We have here diastereomers with two different asymmetric carbon atoms, the spectral behaviour of which may indicate racemoid or mesoid configurations. Figs. 1 and 2 show the absorption curves for the succinic acid derivatives. From the spectrum of the high-melting isomer (Fig. 1) we note that the carbonyl stretching frequency is found at 5.91μ , whereas the low-melting acid (Fig. 2) has one band at 5.82μ and a second at 5.89μ . Moreover, it seems reasonable to ascribe the strong band at 7.83μ in Fig. 1 to the carbon-oxygen stretching of the carboxylic groups (cf. 5). The low-melting isomer has in this region a set of bands, which makes an assignment difficult. The strong bands commonly ascribed to out of plane bendings of OH (6) are found for the high-melting acid at 10.50μ and for the stereoisomer probably as a double peak at 10.70μ and 11.2μ respectively. The exact wavelengths for the absorption bands are collected in Table 1.

When comparing the infrared absorption curves of the diastereomers of α -ethyl- α' -methyl-glutaric acid it can be seen from Figs. 3 and 4 that the spectra are rather similar, even if differences occur in the finger print region (7.5 – 10μ). The high-melting isomer has the carbonyl stretching frequency at 5.88μ as a single top with a slight shoulder at 5.84μ (Fig. 3) and the corresponding peak for the low-melting acid (Fig. 4) is found at 5.85μ . It may further be noted that the OH bending is found for the high-melting acid at 10.70μ , whereas the isomer is probably characterized by two peaks at 10.78μ and 10.95μ . The wavelengths are reported in Table 2.

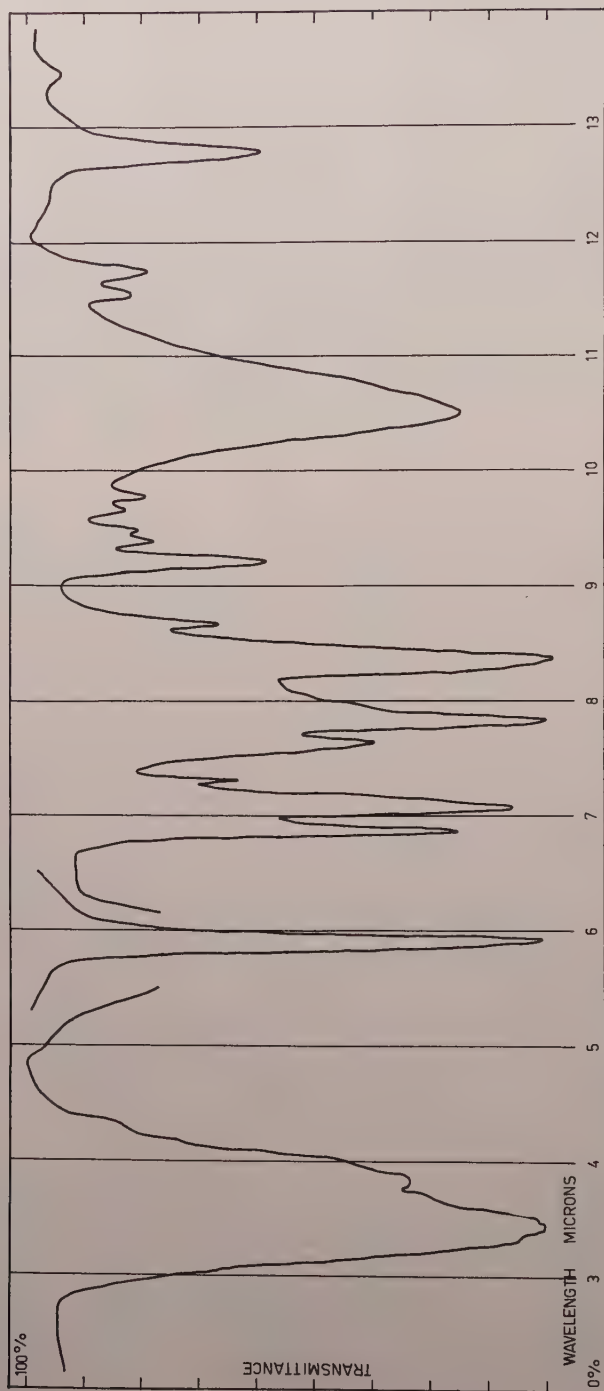


Fig. 1. IR absorption curve of α -ethyl- α' -methyl-succinic acid (high-melting form). KBr phase.

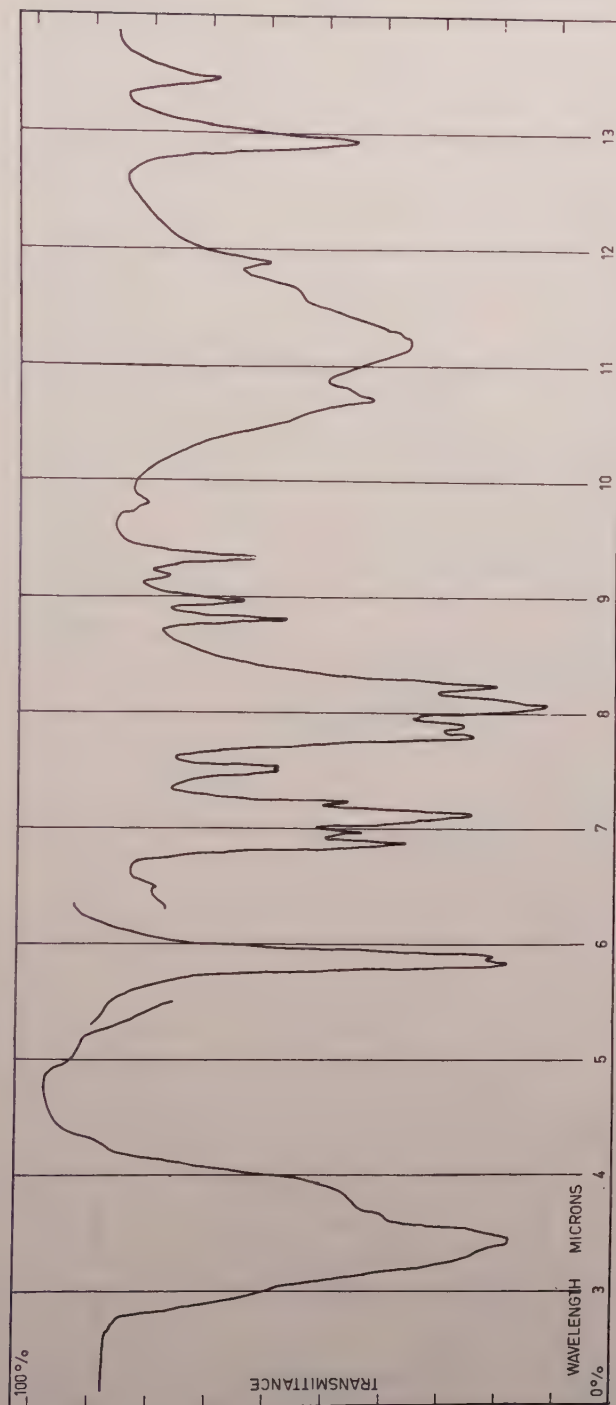


Fig. 2. IR absorption curve of α -ethyl- α' -methyl-succinic acid (low-melting form). KBr phase.

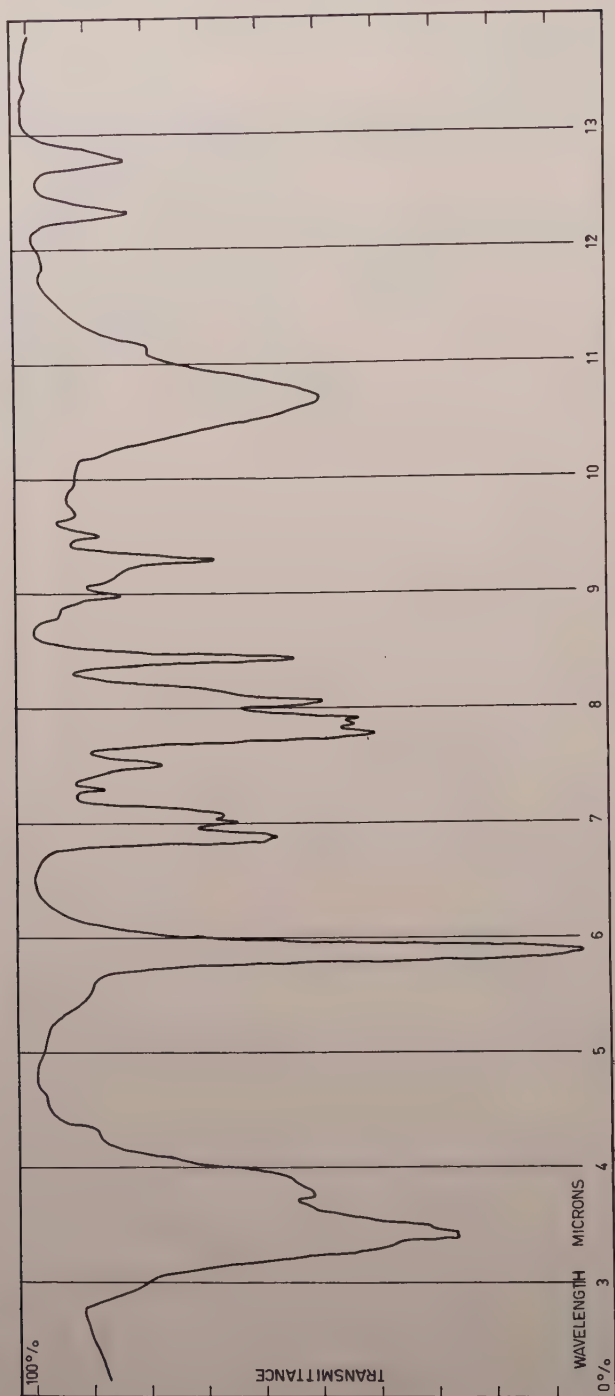


Fig. 3. IR absorption curve of α -ethyl- α' -methyl-glutaric acid (high-melting form). KBr phase.

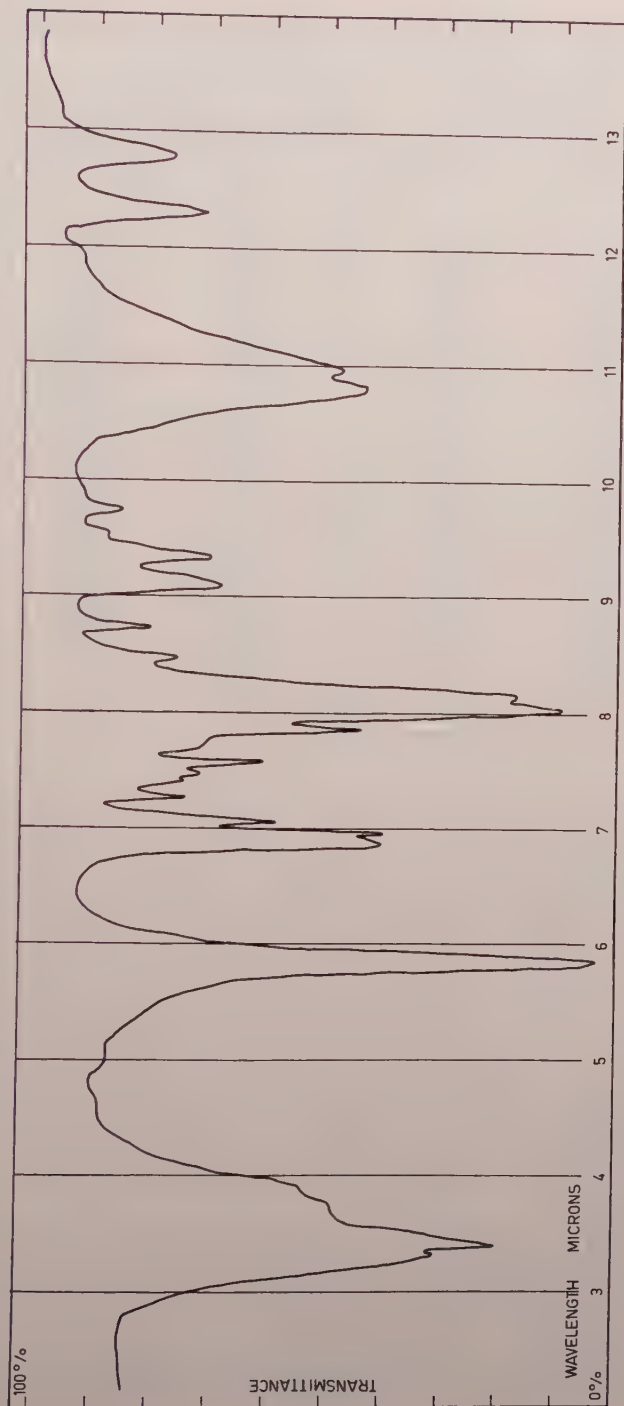


Fig. 4. IR absorption curve of α -ethyl- α' -methyl-glutaric acid (low-melting form). KBr phase.

Table 1. The infrared absorption bands of α -ethyl- α' -methyl-succinic acids.

High-melting form		Low-melting form	
Wavelength in μ	Wavenumber in cm^{-1}	Wavelength in μ	Wavenumber in cm^{-1}
3.44	2907	3.42	2924
3.82	2618	3.8	2632
5.91	1692	5.82	1718
6.86	1458	5.89	1698
7.07	1414	6.87	1456
7.30	1370	6.96	1437
7.64	1309	7.12	1404
7.83	1277	7.22	1385
8.37	1195	7.48	1337
8.66	1155	7.53	1328
9.20	1087	7.79	1284
9.39	1065	7.88	1269
9.48	1055	8.06	1241
9.65	1036	8.22	1217
9.78	1022	8.80	1136
10.50	952	8.96	1116
11.54	867	9.17	1091
11.74	852	9.33	1072
12.77	783	9.80	1020
13.46	743	10.70	935
		11.2	893
		11.88	842
		12.92	774
		13.46	743

For an interpretation of the spectral behaviour of the acids attention is directed to the general discussion given in (4) by SCHOTTE and ROSENBERG. The splitting of the carbonyl stretching frequency into double peaks for the low-melting form of α -ethyl- α' -methyl-succinic acid thus indicates a racemoid configuration, as *racem* α, α' -dimethyl-, α, α' -dichloro- and α, α' -dibromosuccinic acid in contrast to the *meso* isomers are characterized by double peaks for this stretching. The effect on the specific absorption is of about the same order of magnitude as that found for the α, α' -dimethyl derivative. The conclusion drawn above about the configuration seems to be supported by the work of BERNER and LEONARDESEN (7) who found an appreciably higher specific rotation for the antipodes of the low-melting α -ethyl- α' -methyl-succinic acid than for those of the diastereomer (the same alkaloid used for resolution). All the low-melting acids investigated within the succinic acid series thus possess a *trans* configuration.

On the other hand, no assignments can be made about the diastereomers of α -ethyl- α' -methyl-glutaric acid from the spectra reported in Figs. 3 and 4. Both the acids show single peaks in the carbonyl stretching region when examined with a sodium chloride prism. It is already known that the effects to be studied are considerably less pronounced (if at all observable) in the glutaric acid series except for the α, α' -diethyl derivative (4). Thus, here also no conclusions can be drawn from the corresponding spectra of *racem* and *meso* α, α' -dimethylglutaric acid. As even small

Table 2. The infrared absorption bands of α -ethyl- α' -methyl-glutaric acids.

High-melting form		Low-melting form	
Wavelength in μ	Wavenumber in cm^{-1}	Wavelength in μ	Wavenumber in cm^{-1}
3.42	2924	3.32	3012
3.77	2653	3.40	2941
5.88	1701	5.85	1709
6.87	1456	6.87	1456
7.00	1429	6.95	1439
7.07	1414	7.05	1418
7.30	1370	7.27	1376
7.50	1333	7.40	1351
7.77	1287	7.47	1339
7.85	1274	7.58	1319
7.90	1266	7.85	1274
8.05	1242	8.03	1245
8.41	1189	8.15	1227
8.98	1114	8.47	1181
9.28	1078	8.73	1145
9.50	1053	9.09	1100
9.7	1031	9.33	1072
10.70	935	9.54	1048
11.15	897	9.75	1026
11.88	842	10.78	928
12.32	812	10.95	913
12.77	783	12.30	813
		12.78	782

effects, undetectable in the NaCl prism spectra, can be studied with higher resolution, the wavelength region in question was investigated with a calcium fluoride prism. Even more detailed knowledge about an absorption band is obtained, if the spectrum is recorded with the substance in a low-temperature cell (cf. 8). It must, however, be remembered that small changes in the packing of the crystal lattice may be responsible for possible shifts. Care in interpretation should therefore be exercised. When examining the diastereomers of α -ethyl- α' -methyl-glutaric acid for the carbonyl stretching frequency with a CaF_2 prism a splitting of the band for the high-melting acid into two peaks at 5.82μ and 5.88μ is observed even at room temperature, whereas the low-melting isomer only shows a single peak at the frequency given above for the NaCl prism region. This spectral difference for the acids is still more definite at low temperature (-170° ; reached with liquid air), as the splitting of the band is more distinct. A slight shift of the first peak to 5.83μ occurs also. Although the effects are small and only detectable under special conditions we may conclude that the spectral behaviour at least indicates a racemoid configuration for the high-melting α -ethyl- α' -methyl-glutaric acid. Such a conclusion is in good agreement with the results of RYDON (9), who assigned to the low-melting isomer a cis-structure. From the spectral point of view the behaviour of α -ethyl- α' -methyl-glutaric acid is very interesting if compared with that of α,α' -diethylglutaric acid, where the racemic form is the low-melting acid. The explanation given for the behaviour of the carbonyl bands (4), involving coupling between the stretching vibrations of the different carbo-

nyl groups within the molecule, implies that the effects observed should be independent of the melting point order for the diastereomers. This is verified by the above result.

Moreover, it may be noted that the correlation between melting point and configuration for the diastereomers of α -ethyl- α' -methyl-glutaric acid follows that of the α,α' -dimethyl derivative and is opposite to that of α,α' -diethylglutaric acid. Finally, attention is drawn to the fact that an infrared spectrum of *cis* α -ethyl- α' -methyl-glutaric acid has been mentioned earlier in the literature (10). There were, however, no data given which allow a comparison and furthermore the sample used had an appreciably lower melting point than that found in this investigation and in (11).

Experimental

For the *infrared absorption measurements* (wavelength region 2–14 μ) a Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prism was used. The substances were examined as solid pressed potassium bromide plates (12). For a closer study of the carbonyl bands a calcium fluoride prism was inserted. In addition, a low-temperature investigation was made using a technique due to ROSENBERG (8). The calibration of the absorption curves, linear in wavelength is correct to $\pm 0.01 \mu$.

α -Ethyl- α' -methyl-succinic acid (high-melting form).—M.p. 177°–178°. The acid was obtained according to BERNER and LEONARDESEN (7) and recrystallized three times from water.

Anal. Subst., 41.26 mg; 7.44 ml of 0.0696N NaOH. Equ. wt. Calc. 80.1; Found 79.7.

α -Ethyl- α' -methyl-succinic acid (low-melting form).—M.p. 100°–101°. From a mixture of the diastereomers the acid could be separated by means of the neutral calcium salts (7, 13). Five recrystallizations of the liberated acid yielded a pure substance.

Anal. Subst., 41.09 mg; 7.33 ml of 0.0696N NaOH. Equ. wt. Calc. 80.1; Found 80.5.

α -Ethyl- α' -methyl-glutaric acid (high-melting form).—M.p. 104°5–105°5. The acid is obtained from a mixture of the diastereomers, prepared as described in (9) or (11), through the acid calcium salt (11). The acid was eventually recrystallized several times from water.

Anal. Subst., 34.11 mg; 5.61 ml of 0.0696N NaOH. Equ. wt. Calc. 87.1; Found 87.4.

α -Ethyl- α' -methyl-glutaric acid (low-melting form).—M.p. 83°5–84°5. The substance obtained from the above mother liquor of the acid calcium salt (after acidification) was recrystallized three times from small amounts of water. — Appreciably lower melting points for this acid have been reported earlier (9, 10, 14).

When the acid of m.p. 83°5–84°5 was mixed with that of m.p. 104°5–105°5 a considerable melting point depression was observed.

Anal. Subst., 20.80 mg; 3.43 ml of 0.0696N NaOH. Equ. wt. Calc. 87.1; Found 87.1.

Summary

The infrared absorption curves of the diastereomers of α -ethyl- α' -methyl-succinic and α -ethyl- α' -methyl-glutaric acid have been investigated.

The spectral behaviour of the acids resembles that of the earlier investigated meso and racemic forms of some succinic and glutaric acid derivatives.

The differences observed seem to have given information about the configurations of the acids.

ACKNOWLEDGEMENTS

The author is indebted to Professor ARNE FREDGA for his stimulating interest in this work. Samples of the glutaric acid derivatives, prepared by Dr. GERD BENDZ (11), were kindly placed at my disposal.

The author also wishes to express his gratitude to Dr. ANDREAS ROSENBERG for valuable discussions and to Mr. STIG BERGWALL for experimental help with the infrared work.

Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, November 1955.

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Tryckt den 12 mars 1956

Uppsala 1956. Almqvist & Wiksells Boktryckeri AB



The sterical isomers of α, α' -dibromopimelic acid

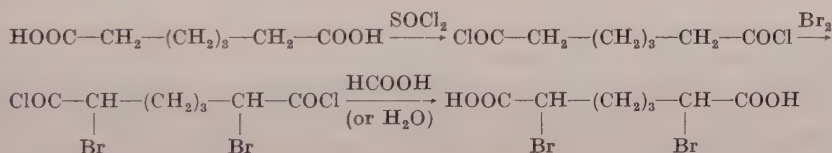
By LENNART SCHOTTE

With 2 figures in the text

The α, α' -dibromination of pimelic acid was first described by WILLSTÄTTER in 1895 (1). Only one compound, m.p. 140° – 142° , was isolated and the steric configuration was not discussed. Independently of each other FEHNEL and OPPENLANDER (2) and the present author (3, 4) reinvestigated the bromination of pimelic acid in an attempt to obtain stereochemically pure starting materials for the preparation of cyclic sulphides and disulphides.

FEHNEL and OPPENLANDER slightly modified the original procedure of WILLSTÄTTER and isolated in a pure state the high-melting acid, m.p. 155° , to which they ascribed the meso configuration. In spite of several experiments they failed to isolate the low-melting isomer from the mother liquors. Only waxy solids with wide melting point ranges could be isolated and equivalent weight determinations indicated contamination with the monobromo acid. It was, however, observed that the formic acid mother liquors upon standing for a long time slowly deposited further quantities of the high-melting dibromo acid.

The present author also used the general method for preparation of α -halogeno substituted dicarboxylic acids, i.e.



The hydrolysis of the acid chloride was first made in water (cf. 5). Data in good agreement with those reported by FEHNEL and OPPENLANDER were obtained for the high-melting acid. This isomer in reaction with sodium sulphide gave *cis*-(*meso*)-2,6-tetrahydrothiapyrandicarboxylic acid (4, cf. 2). Starting from an α, α' -dibromopimelic acid with m.p. 136° – 144° , on the other hand, a mixture of the diastereomers of the sulphide acid was prepared, from which the *trans*-(*racem*)-form could be separated by means of the calcium salts. This indicates the presence of both the diastereomers in the acid of m.p. 136° – 144° , as the substitution in this case is likely to proceed without *racem* $\rightleftharpoons$ *meso* rearrangements (6, 7).

Now, it seemed justified to try to find a separation method for the diastereomers of α, α' -dibromopimelic acid. It was then at first established that the brominated acid chloride was best hydrolyzed with formic acid according to FEHNEL and OPPEN-

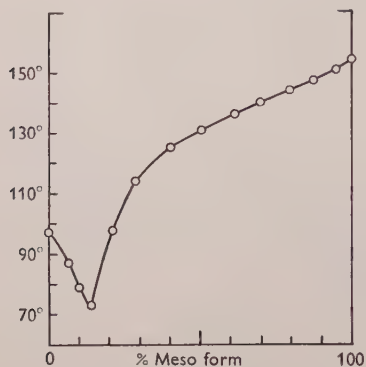


Fig. 1. Melting point diagram for mixtures of the diastereomers of α,α' -dibromopimelic acid.

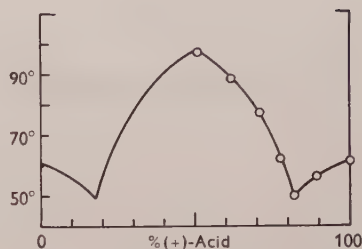


Fig. 2. Melting point diagram for the system (+)- and (-)- α,α' -dibromopimelic acid.

LANDER. Their experimental report has been verified, but it has been found advisable to double the time for the addition of the bromine. In one of the experiments a low-melting fraction was obtained, which upon recrystallization yielded an acid with m.p. 95° – 96° . This low-melting acid showed an appreciable melting point depression when mixed with the high-melting acid. The melting point could not be raised in spite of several recrystallizations (also some from formic acid). Preliminary resolution experiments indicated that this acid might have the racemic configuration. However, on that occasion the conditions must have been especially favourable since the result could not be reproduced. Previous work has moreover shown (4) that simple fractional crystallization does not yield both the diastereomers in a pure state but only the high-melting form, if proper solvents (as formic acid) are used.

After intensive investigations it was found that a separation of the diastereomers was possible by extraction with cold chloroform (see experimental part). It was also found advisable to reduce the volume of formic acid for the hydrolysis in order to avoid prolonged standing of the mother liquor with gradual conversion of the low-melting form into the isomer as a consequence.

Resolution experiments on the two α,α' -dibromopimelic acids, m.p. 152° – 154° and 95.5° – 97° respectively, have proved that the low-melting isomer possesses the racemic configuration. The acid brucine salt of the high-melting isomer thus yielded an optically inactive acid with unchanged melting point. Under analogous conditions resolution of the low-melting acid was possible. A dextrorotatory acid was obtained from the acid brucine salt, whereas the acid cinchonidine salt yields the antipode. The indications obtained above in the cyclization reactions about the configuration of the high-melting α,α' -dibromopimelic acid thus coincide with the resolution data. The melting point diagram for mixtures of the diastereomers is given in Fig. 1. The diagram is of the eutectic type with the eutectic point found for a mixture of about 15% meso and 85% racemic form.

It is of interest to compare the diastereomers of α,α' -dibromopimelic acid to those of the corresponding α,α' -dimethyl derivative. In both cases the *meso* form is the high-melting acid but whereas the difference in melting point of the bromo compounds is 57° , it is only 5° (8,9) for the diastereomers of α,α' -dimethylpimelic acid. For comparison of the melting points available data for some homologues (from the

Table 1. Melting points of the diastereomers of some homologous α,α' -dimethyl- and α,α' -dibromodicarboxylic acids.

Derivative of	α,α' -Dimethyl-		α,α' -Dibromo-	
	<i>Meso</i> form	<i>Racem</i> form	<i>Meso</i> form	<i>Racem</i> form
Succinic acid	197° (d.)	129°	255°–256°	168°
Glutaric acid	128°	141°	170°; (172°) ¹	142°; (136°) ¹
Adipic acid	144°	76°	191°–193°	143°–144°
Pimelic acid	81°–81.5°	76°–76.5°	152°–154°	95.5°–97°
Suberic acid	— ²	— ²	172°–173°	120°–121°

¹ No conclusive evidence is available for this assignment.² Two isomers, m.p. 132°–133° and 91°–92° resp., have been isolated but no assignments as to the configurations are given.Table 2. Optical activities of some enanthiomorphs of homologous α,α' -dibromodicarboxylic acids. (Only data for one antipode are given.)

α,α' -Dibromo-derivative of	$[\alpha]_D$	$[M]_D$	Solvent used for measurement
Succinic acid	–147.8° ¹⁸	–408°	Ethyl acetate
Glutaric acid	—	—	—
Adipic acid	+66.3° ¹⁸	+202°	Abs. ethanol
Pimelic acid	+57° ²⁵	+180°	Abs. ethanol
„ „	+67° ²⁵	+214°	Glac. acetic acid
„ „	+98° ²⁵	+311°	Ethyl acetate
Suberic acid	—	—	—

literature) are collected in Table 1. Deviations from the apparent regularity are found for the glutaric acid system, where the racemic form of the α,α' -dimethyl derivative is the high-melting isomer. There is no unambiguous evidence as to the configuration of the α,α' -dibromoglutaric acids.

The preliminary resolution of *racem*- α,α' -dibromopimelic acid mentioned above for the configuration determinations of the diastereomers could be accomplished by repeated recrystallizations of the respective salts. No elimination of hydrogen bromide was observed. Acid salts should be used throughout. For the lower homologues difficulties were encountered in the resolution experiments; data for the succinic and adipic acid derivatives are to be found in the literature (10, 11, 12, 5) and for comparison are given in Table 2. In spite of extensive experiments the present author has not succeeded in resolving α,α' -dibromoglutaric acid because of the rapid elimination of hydrogen bromide (obviously due to lactonization). A significant qualitative comparison of the reactivity of the homologous dibromo acids is thus obtained from the resolution experiments. The melting point diagram of the system (+)- and (–)- α,α' -dibromopimelic acid is given in Fig. 2. It is obvious that the enanthiomorphs have a pronounced tendency for racemate formation. The racemic acid is evidently a true racemate and the melting point given above has been verified.

Finally it may be pointed out that the diastereomers of α,α' -dibromo- and α,α' -dimethylpimelic acid have been examined as to their infrared absorption in the sodium chloride prism region. The infrared study of these acids is in line with recent work on various substituted dicarboxylic acids by ROSENBERG and SCHOTTE (13, 14, 15). The bands related to the carbonyl stretching vibrations of the carboxylic groups are found at $5.83\ \mu$ for *meso*- α,α' -dibromopimelic acid and at $5.85\ \mu$ for the racemic isomer. The corresponding values for α,α' -dimethylpimelic acid are $5.83\ \mu$ and $5.84\ \mu$ respectively. No splitting of the carbonyl stretching frequency for the racemic acids into double peaks has been observed, as could be expected from the length of the carbon chain (14).

Experimental

α,α' -Dibromopimelic acid.—A mixture of 67.5 g (0.422 mole) of pimelic acid and 122 g (1.03 mole) of thionyl chloride was heated in a three-necked round-bottomed 250 ml flask with stirring on a water bath until sulphur dioxide and hydrogen chloride were no longer evolved (about 45 minutes). The excess of thionyl chloride was removed *in vacuo*. Bromine (157 g; 0.98 mole) was then added dropwise to the remaining clear solution on the water bath. The addition of bromine required one hour and the mixture was heated one hour more. Continual stirring was applied. The crude brominated acid chloride was (while still hot) poured slowly into 80 ml of hot formic acid (about 15 minutes), which caused immediate vigorous evolution of hydrogen chloride and carbon monoxide. In order to complete the hydrolysis the reaction mixture was refluxed for another 15 minutes. The formic acid solution was allowed to stand at room temperature for three hours, after which the separated product was filtered. 82 g (61%) with m.p. about 120° – 140° was obtained. The mother liquor was then placed in a refrigerator and after one day another 12 g (8.9%) had separated, m.p. 110° – 125° . The solution was again placed in the refrigerator and the crystals obtained filtered each day. After about a week a total of 17 g (13%) had crystallized with melting points between 70° and 85° .

For the isolation of the *meso form* the combined first two fractions were shaken with 300 ml of cold chloroform during 2–3 minutes, which gave 67 g (50%) with m.p. 136° – 145° as a residue. Two recrystallizations of this acid from formic acid yielded 40 g (30%) of *meso*- α,α' -dibromopimelic acid, m.p. 150° – 153° . After repeated recrystallizations from the same solvent an acid with constant m.p. 152° – 154° was obtained. It consisted of colourless prisms.

Anal. Subst., 106.28 mg; 9.06 ml of 0.0733*N* NaOH. Equ. wt. Calc. 159.1; Found 160.0.

For the isolation of the *racemic form* the chloroform solution from above was evaporated to give 25.5 g (19.0%) of dry residue with m.p. about 75° . This acid was now combined with the above mentioned low-melting fractions and the treatment with cold chloroform repeated. 12 g (8.9%) of acid with m.p. 90° – 100° (the separation could be continued on this fraction) remained undissolved, while the chloroform extract upon evaporation yielded 29 g (21.5%) of bromo acid, m.p. 80° – 85° . Three recrystallizations of this acid from toluene gave 15.2 g (11.3%) of *racem*- α,α' -dibromopimelic acid, m.p. 89° – 94° (still to a slight degree contaminated with the diastereomer). Another three recrystallizations, now from small amounts of formic acid, eventually yielded an acid (colourless prisms) with m.p. 95.5° – 97° .

Anal. Subst., 26.45 mg; CO₂, 25.48 mg; H₂O, 7.20 mg.
 .. , 26.45 mg; 8.50 ml of 0.01956N Hg(NO₃)₂.
 .. , 97.10 mg; 8.27 ml of 0.0733N NaOH.

C₇H₁₀O₄Br₂ (318.15) Calc. C 26.42; H 3.17; Br 50.29; Equ. wt. 159.1
 Found C 26.29; H 3.05; Br 50.29; Equ. wt. 160.2.

Racem- α,α' -dibromopimelic acid shows appreciably greater solubility than the meso isomer in common organic solvents.

In one experiment with the same amounts as above (but with 100 ml of formic acid for hydrolysis) the mother liquors deposited 12 g (8.9%) of an acid with m.p. 91°–95° after the separation of high-melting fractions. This acid could be recrystallized to give the pure racemic isomer. The result could, however, not be reproduced.

Resolution of racem- α,α' -dibromopimelic acid.—9.7 g (0.0305 mole) of racemic acid and 8.9 g (0.030 mole) of cinchonidine were dissolved in 200 ml of hot acetone with addition of 20 ml of water. The solution was allowed to cool and then placed in the refrigerator. After one week the precipitate was filtered off, dried and weighed. About 0.3 g was taken for preliminary measurement of the optical activity (on the liberated acid) and the rest recrystallized from acetone, to which was added small amounts of water. The results are given below, where the optical activity of the acid is somewhat approximate, as the liberated acid was not purified before measurement.

Crystallization	1	2	3
ml of acetone	200	100	70
ml of water	20	5	3
Salt obtained, in g	4.2	2.9	1.9
$[\alpha]_D^{25}$ of acid (abs. ethanol)	–45°	–54°	–56°

The acetone was removed from the above first mother liquor and the remaining acid liberated. It was dissolved together with 10.5 g of brucine (4 H₂O) (0.023 mole) in a hot mixture of 85 ml of acetone and 85 ml of water. The solution was left at room temperature and filtered after one day. The salt obtained was then recrystallized until the optical activity of the liberated acid remained roughly constant.

Crystallization	1	2	3	4
ml of acetone	85	75	50	35
ml of water	85	75	50	35
Salt obtained, in g	10	6.0	3.9	2.4
$[\alpha]_D^{25}$ of acid (abs. ethanol)	—	+46°	+59°	+57°

It may be noted here that no salts were obtained in alcohol or acetone with quinine, strychnine and cinchonine.

(+)- α,α' -Dibromopimelic acid.—The acid brucine salt was decomposed with 4N sulphuric acid and the liberated acid was extracted with ether. After evaporation of the solvent an oily residue was obtained, which crystallized *in vacuo* after scratching the sides of the walls with a glass-rod. The optically active acid was recrystallized from a mixture of ether and light petroleum (b.p. 40°–60°) and obtained as rectangular transparent prisms with m.p. 60°–61°.

Due to the low melting point and the solubility conditions of the acid appreciable difficulties were encountered in the recrystallizations; hence only small amounts of the pure components were obtained.

Anal. Subst., 66.19 mg; 5.74 ml of 0.0721N NaOH. Equ. wt. Calc. 159.1; Found 159.9.

The optical activity of the dextrorotatory acid was measured in three different solvents (10.00 ml). The results are given below.

Solvent	mg of acid	$2 \alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Abs. ethanol	66.2	+ 0.75°	+ 57°	+ 180°
Glacial acetic acid	34.2	+ 0.46°	+ 67°	+ 214°
Ethyl acetate	54.2	+ 1.06°	+ 98°	+ 311°

(-)- α, α' -Dibromopimelic acid.—The acid was liberated from the acid cinchonidine salt as described above for the enanthiomorph. The levorotatory acid resembled its antipode. M.p. 59°–61°.

Anal. Subst., 38.51 mg; 3.34 ml of 0.0721N NaOH. Equ. wt. Calc. 159.1; Found 159.9.

38.5 mg of the acid in 10.00 ml of abs. ethanol: $2 \alpha_D^{25} = -0.43^\circ$; $[\alpha]_D^{25} = -56^\circ$; $[M]_D^{25} = -178^\circ$.

For the *infrared absorption measurements* a Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prism was used. The acids were examined as solid pressed potassium bromide plates. The wavelengths are correct to $\pm 0.01 \mu$.

The preparations for the *melting point diagrams* were made by dissolving exactly weighed amounts of the components in acetone of high purity. The solvent was evaporated at room temperature and the residues dried *in vacuo* after powdering. The melting points were determined in capillary tubes (calibrated thermometer; electrically heated apparatus).

Summary

Meso- and *racem*- α, α' -dibromopimelic acid (m.p. 152°–154° and 95.5°–97° resp.) have been prepared and a separation method is described.

Optical resolution of the low-melting acid has provided conclusive evidence as to the configurations.

The enanthiomorphs have been characterized.

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The author is indebted to Professor ARNE FREDGA for valuable discussions.

The infrared curves have been recorded by Mr. STIG BERGWALL.

Thanks are also due to Mr. HOLGER STRÖM for skilful help with parts of the experimental work.

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Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, December 1955.

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Uppsala 1956. Almqvist & Wiksells Boktryckeri AB

α,α' -Dimercaptopimelic acid and related compounds

By LENNART SCHOTTE

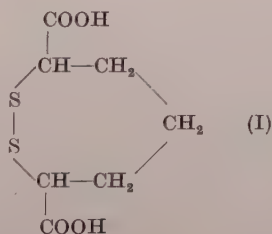
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In 1938 FREDGA investigated the steric isomers of α,α' -dimercaptoadipic acid and its cyclic disulphides (1, 2). The preparation of α,α' -dimercaptosuccinic acid was later reported by OWEN och SULTANBAWA although with no separation of the stereoisomeric forms (3). The present author has published preliminary reports on the cyclic disulphides related to glutaric and pimelic acid (4, 5).

Both the diastereomers of α,α' -dibromopimelic acid have recently been characterized (6), thus making it possible to complete the investigation of the pimelic acid series. Although there are several methods for the preparation of mercapto acids (7), there are two methods which are generally used: the reduction of the corresponding disulphides and the treatment of the xanthogen substituted acids with ammonia.

The two diastereomers of α,α' -bis-ethylxanthogen pimelic acid were obtained without complications from the corresponding dibromo acids. The dimercapto acids could then be prepared in the usual manner. It may be noted here that *racem*- α,α' -dimercaptopimelic acid is the high-melting isomer in contrast to the case for the dibromo and bis-ethylxanthogen substituted compounds. The dimercapto acids have also been obtained by reduction (2) of the corresponding disulphides (see below). The configurations of the mercapto acids are thus incontrovertibly settled.

The mercapto groups have been characterized in reactions with 2,4-dinitrochlorobenzene giving bis-thioethers. Attempts to prepare the dibenzyl derivatives yielded only oily products.



Meso- and *racem*-1,2-dithiepane-3,7-dicarboxylic acid (1,2-dithiacycloheptane-3,7-dicarboxylic acid) (I) have been prepared from the diastereomers of α,α' -dibromopimelic acid and sodium disulphide and by oxidation of the corresponding mercapto acids. The racemic disulphide acid has also been obtained by rearrangement of the meso isomer.

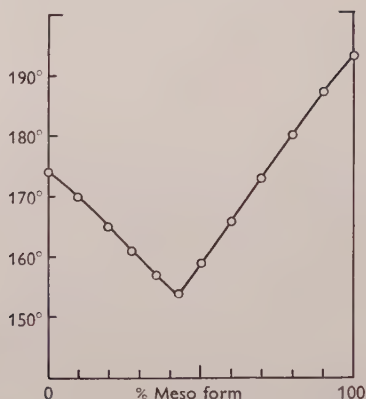


Fig. 1. Melting point diagram for mixtures of the diastereomers of 1,2-dithiepane-3,7-dicarboxylic acid.

For the adipic acid series FREDGA has found that the sodium disulphide reactions give both the cyclic disulphide and sulphide, the latter with separation of sulphur (8). Analogous behaviour has also been observed in this investigation, where in the case of the meso isomer the six-membered sulphide acid (9) could be isolated in small amounts from the mother liquor of the 1,2-dithiepane derivative. These observations are interesting as they seem to elucidate rather well the nature of the replacements in cyclization reactions. A comparison of the two cases should also include the fact that considerable amounts of polymeric products (probably of linear structure with indefinite composition) are formed in sodium disulphide reactions with α,α' -dibromopimelic acid (5). As could be expected the relative amount of polymer has been found to vary with the concentration of the reaction mixture. The yield of monomeric disulphide dicarboxylic acid is thus appreciably improved at high dilution. No polymer formation is found for the adipic acid series (2, 8), where the related cyclic disulphide acid is six-membered.

The oxidation of α,α' -dimercaptopimelic acid to the disulphide has been performed with hydrogen peroxide, oxygen (cf. 10) and iodine. The relative difficulty in obtaining the seven-membered ring system in good yield is further pointed out here as the oxidations, in spite of high dilution, also give polymers besides the 1,2-dithiepane derivatives. The lower homologue, α,α' -dimercaptoadipic acid, yields the monomeric disulphide without complications (1, 2). For the pimelic acid series iodine has proved to be the best oxidizing agent. It is of great importance to note that in the iodine and oxygen oxidation experiments on *meso*- α,α' -dimercaptopimelic acid a high-melting product could be isolated (very small amounts), the composition and equivalent weight of which is in accordance with that expected for a 1,2-dithiepane derivative. The related dimercapto acid could be regained in reduction with zinc powder in ammoniacal solution. The disulphide substance (m.p. 235°–237° (d.)) has, however, a polymeric appearance and shows a very low solubility in common solvents, in contrast to the meso acid (m.p. 192°–193°) with proved monomeric structure. Due to the solubility properties no exact molecular weight determinations were possible, but the attempts indicate a polymeric nature. It should be mentioned that the formation of this polymer seems connected with the oxidation process itself and not with the separation and purification procedure of the monomer, since experiments on

pure *meso*-1,2-dithiepane-3,7-dicarboxylic acid have proved its stability under the conditions employed. The result suggests that a characterization of cyclic disulphide acids with large rings might be possible under special conditions.

Oxidation experiments on α,α' -dimercaptosuberic acid give only polymeric products (11).

It is very tedious work to prepare *racem*-1,2-dithiepane-3,7-dicarboxylic acid in some quantity from the corresponding dibromo acid, but a valuable alternative is the partial conversion of the *meso* isomer by melting and heating for some minutes at about 200°, even if the yield of pure racemic acid is small. The present author has used this type of racemization process in the case of 2,6-tetrahydrothiapyrandicarboxylic acid also (9). In these two cases, where the racemic isomers are the low-melting acids, a mixture of the diastereomers is obtained, from which the pure acids can be isolated. A complete conversion of the *cis*-(*meso*)-isomer into the diastereomer was realized by FREDGA (2) in the adipic acid series. The high-melting *trans*-(*racem*)-disulphide acid crystallizes from the melt, thus making a complete rearrangement possible.

A confirmation of the configurations of the 1,2-dithiepane acids has been obtained by preliminary resolution experiments. The acid brucine salt of the low-melting acid in acetone-water gives the (+)-form, whereas the neutral brucine salt yields the antipode. In this respect the acid resembles exactly *racem*-1,2-dithiolane-3,5-dicarboxylic acid (4). Lack of substance has unfortunately made a complete resolution impossible, but rather high rotatory power is already indicated (cf. 12). The acid and neutral brucine salts of the high-melting disulphide acid yield only inactive acids with unchanged melting points. The same result is obtained with the acid strychnine and the neutral quinine salts. The high-melting 1,2-dithiepane-3,7-dicarboxylic acid thus possesses the *meso* configuration.

The melting point diagram between *meso*- and *racem*-1,2-dithiepane-3,7-dicarboxylic acid is given in Fig. 1. It is of the common eutectic type with the eutectic point at a composition of 43 % *meso* form and 57 % racemic form.

Experimental

Meso- α,α' -bis-ethylxanthogen pimelic acid.—19.6 g (0.0616 mole) of *meso- α,α' -dibromopimelic acid* was suspended in 60 ml of water. 17.6 g (0.0615 mole) of sodium carbonate (10 H₂O), dissolved in 60 ml of water, was added slowly with cooling. Solid potassium ethyl xanthate (22.7 g; 0.142 mole) was added immediately and the mixture shaken until homogenous. After standing at room temperature for three days, the solution was acidified with dilute sulphuric acid. The bis-xanthogen acid separated as an oil which soon solidified. The yield of crude acid was 17.3 g (70.0 %) with m.p. 128°–132°. The substance was recrystallized twice from formic acid to eventually yield 15.3 g (61.9 %) of the pure acid. M.p. 136°–137.5°.

If the reaction time is made shorter a lower yield is obtained and the product crystallizes less readily.

Anal. Subst., 11.06 mg; 6.61 ml of 0.0334*N* NaOH.

„ , 45.01 mg; 6.75 ml of 0.0334*N* NaOH.

C₁₃H₂₀O₆S₄ (400.53) Calc. S 32.02; Equ. wt. 200.3.

Found S 32.00; Equ. wt. 199.6.

Racem- α, α' -bis-ethylxanthogen pimelic acid.—The same procedure was used as described above for the *meso* isomer. Starting from 12.0 g (0.0377 mole) of *racem- α, α' -dibromopimelic acid* 12.4 g (82.1 %) of crude bis-ethylxanthogen acid (m.p. 109°–112°) was obtained. Recrystallized twice from formic acid the product was pure (8.5 g; 56 %) and showed m.p. 114°–115°.

Anal. Subst., 25.50 mg; CO₂, 36.28 mg; H₂O, 11.34 mg.

„ , 14.42 mg; 4.04 ml of 0.0721N NaOH.

„ , 94.30 mg; 6.56 ml of 0.0721N NaOH.

C₁₃H₂₆O₆S₄ (400.53) Calc. C 38.98; H 5.03; S 32.02; Equ. wt. 200.3.

Found C 38.82; H 4.98; S 32.38; Equ. wt. 199.4.

Meso- α, α' -dimercaptopimelic acid

A. From meso- α, α' -bis-ethylxanthogen pimelic acid.—15.3 g (0.0382 mole) of *meso- α, α' -bis-ethylxanthogen pimelic acid* was dissolved in 100 ml of concentrated ammonia and the solution left for three days in a well-filled stoppered flask. The ammonia was then removed at reduced pressure (nitrogen atmosphere) while the temperature was kept between 20° and 30°. The remaining neutral solution was made alkaline with an aqueous solution of sodium carbonate and the xanthogen amide formed removed by extraction with ether. After acidification (2N H₂SO₄) of the water solution the dimercaptopimelic acid was extracted with ether (six times). The ether solution was dried with anhydrous sodium sulphate and the ether evaporated at reduced pressure (nitrogen). 7.9 g (92 %) of crude product (m.p. 71°–80°) was obtained. Three recrystallizations from benzene (small volumes) gave pure *meso- α, α' -dimercaptopimelic acid* (colourless needles) with m.p. 81°–83°. The yield was 6.7 g (78 %).

Anal. Subst., 25.61 mg; CO₂, 35.01 mg; H₂O, 11.65 mg.

„ , 28.77 mg; 7.14 ml of 0.0721N NaOH.

„ , 62.03 mg; 7.52 ml of 0.0733N NaOH.

„ , 93.96 mg; 8.40 ml of 0.0999N J₂.

C₇H₁₂O₄S₂ (224.29) Calc. C 37.48; H 5.39; S 28.59; Equ. wt. 112.1.

Found C 37.29; H 5.08; S 28.68; Equ. wt. 112.5; 112.0.

B. From meso-1,2-dithiepane-3,7-dicarboxylic acid.—1.5 g (0.0067 mole) of *meso-1,2-dithiepane-3,7-dicarboxylic acid* was dissolved in 15 ml of 4N ammonia and the solution sucked through a layer of zinc powder four times. The solution was then neutralized with dilute sulphuric acid and the separated zinc mercaptide immediately suspended in dilute sulphuric acid to liberate the dimercaptopimelic acid. Extraction with ether, followed by the procedure as above, gave after one recrystallization from benzene 1.2 g (80 %) of *meso- α, α' -dimercaptopimelic acid*, m.p. 82°–83°. Mixed m.p. (substance from A and B) showed no depression.

Racem- α, α' -dimercaptopimelic acid

A. From racem- α, α' -bis-ethylxanthogen pimelic acid.—The same procedure was used as described above for the *meso* isomer. Thus 7.7 g (0.019 mole) of *racem- α, α' -bis-ethylxanthogen pimelic acid* gave 3.4 g (79 %) of crude dimercapto acid, m.p. 85°–95°. Two recrystallizations from benzene yielded pure racemic acid (2.4 g; 55.5 %) with m.p. 106°–107°.

Anal. Subst., 33.43 mg; CO₂, 46.12 mg; H₂O, 16.01 mg.

.. , 20.30 mg; 4.98 ml of 0.0721N NaOH.

.. , 50.35 mg; 6.25 ml of 0.0721N NaOH.

.. , 65.27 mg; 5.79 ml of 0.0999N J₂.

C₇H₁₂O₄S₂ (224.29) Calc. C 37.48; H 5.39; S 28.59; Equ. wt. 112.1.

Found C 37.66; H 5.35; S 28.35; Equ. wt. 111.7; 112.8.

B. From racem-1,2-dithiepane-3,7-dicarboxylic acid.—Reduction with zinc in ammoniacal solution as above (the meso isomer: *B*) of 0.30 g (0.00135 mole) of *racem*-1,2-dithiepane-3,7-dicarboxylic acid gave 0.25 g (83 %) of dimercapto acid, m.p. 90°–100°. Recrystallized from benzene twice the acid showed m.p. 105°–107°. Mixed m.p. (substance from *A* and *B*) showed no depression.

Racem-α,α'-dimercaptopimelic acid is soluble in water and common organic solvents but shows a considerably lower solubility than the meso acid. From benzene the racemic isomer is obtained as colourless needles.

Bromothymol blue has been used as an indicator in the titrations of the dimercapto acids.

Meso-α,α'-bis-(2,4-dinitrophenyl-mercapto)-pimelic acid

0.86 g (0.0038 mole) of *meso-α,α'*-dimercaptopimelic acid was completely neutralized with an aqueous solution (40 ml) of 0.86 g (0.015 mole) of potassium hydroxide. 1.55 g (0.00764 mole) of 2,4-dinitrochlorobenzene in 40 ml of alcohol was added and the mixture left at room temperature over-night. After evaporation of the alcohol the residue was acidified with dilute sulphuric acid. The crude product (2.0 g; 94 %) was recrystallized twice from alcohol and after removal of a non-acidic fraction obtained as long yellow needles. M.p. 55° (preheated apparatus). The melting point depends on the method of determination; the substance seems to rearrange on heating.

Anal. Subst., 85.47 mg; 4.18 ml of 0.0721N NaOH. Equ. wt. Calc. 278.2; Found 283.6.

The colour of the solutions made it somewhat difficult to determine the equivalent weight accurately by the indicator method.

Racem-α,α'-bis-(2,4-dinitrophenyl-mercapto)-pimelic acid

0.30 g (0.00135 mole) of *racem-α,α'*-dimercaptopimelic acid gave 0.7 g (93 %) of crude product using a procedure analogous to that described above. Two recrystallizations from alcohol gave the pure acid with m.p. 68°–70° as yellow needles.

Anal. Subst., 81.16 mg; 4.00 ml of 0.0721N NaOH. Equ. wt. Calc. 278.2; Found 281.4.

Meso-1,2-dithiepane-3,7-dicarboxylic acid

A. From meso-α,α'-dibromopimelic acid

31.8 g (0.10 mole) of *meso-α,α'*-dibromopimelic acid was rapidly neutralized with an aqueous solution (200 ml) of 16.8 g (0.20 mole) of sodium bicarbonate. A solution of sodium disulphide (0.12 mole) in 300 ml of water (prepared from Na₂S·9H₂O and sulphur in water 48 hours before use) was added. The total volume was adjusted to

about 700 ml. After 25 hours some precipitated sulphur was filtered off and the solution acidified with dilute sulphuric acid. Polymeric products immediately separated as a viscous oil. The aqueous solution was extracted repeatedly (seven times) with ether and the ether extract was dried with anhydrous sodium sulphate. After spontaneous evaporation of the solvent a colourless crystalline mass remained. The yield of crude product was 16 g (72 %) with m.p. 145° – 170° . The acid is best recrystallized from water (which should be rapidly performed). Two recrystallizations gave 8.5 g (38 %) of *meso*-1,2-dithiepane-3,7-dicarboxylic acid as hard prisms with m.p. 191° – 193° (preheated apparatus).

The melting point depends somewhat on the rate of heating but the melting seems to occur without any decomposition. The influence of the dilution on the yield of monomeric disulphide is obvious from the fact that an analogous experiment with a total volume of 240 ml (cf. 700 ml above) gave only 11 % of 1,2-dithiepane derivative.

Anal. Subst., 16.31 mg; 8.87 ml of 0.0334*N* NaOH.

„ , 24.99 mg; 6.71 ml of 0.0334*N* NaOH.

$C_7H_{10}O_4S_2$ (222.27) Calc. S 28.85; Equ. wt. 111.1.

Found S 29.12; Equ. wt. 111.5.

The molecular weight was determined by the ebullioscopic method with acetone as solvent (cf. 13). Mol. wt. Calc. 222.3; Found 223.

From the mother liquor of the first recrystallization a small amount (0.9 g) of *meso*-2,6-tetrahydrothiapyrandicarboxylic acid (m.p. 208° – 209°) was isolated after extraction with ether and fractional crystallization of the residue (separation is also possible by means of the calcium salts). The sulphide acid was identified with an authentic sample.

B. By oxidation of meso- α,α' -dimercaptopimelic acid

a. With hydrogen peroxide.—1.5 g (0.0067 mole) of *meso- α,α' -dimercaptopimelic acid* was dissolved in 175 ml of water and an aqueous solution (175 ml) of 1.2 g (excess) of sodium bicarbonate added with cooling. A few drops of ferric chloride was used as an indicator (deep violet colour). 10 % hydrogen peroxide was dropped into the solution slowly (continued cooling) until the violet colour disappeared. The reaction mixture was acidified (4*N* sulphuric acid) and some crystals of sodium sulphite added. Extraction with ether left most of the polymeric products formed undissolved. Upon evaporation of the solvent (after drying) 0.45 g (30 %) of crude product (not quite solid) remained. One recrystallization from water gave a disulphide acid (0.30 g; 20 %) with m.p. 184° – 188° . Repeated crystallization from water raised the m.p. to 190° – 192° (preheated apparatus).

Anal. Subst., 63.04 mg; 7.72 ml of 0.0733*N* of NaOH. Equ. wt. Calc. 111.1; Found 111.4.

b. With oxygen.—1.5 g (0.0067 mole) of *meso- α,α' -dimercaptopimelic acid* was neutralized as in *a* and oxygen bubbled through the solution until the violet colour ($FeCl_3$ as an indicator and a catalyst) disappeared (about two hours). The purification procedure was performed as above. 1.0 g (67 %) of crude product was obtained; m.p. about 175° . Upon recrystallization from water 0.15 g remained undissolved and 0.45 g (30 %) crystallized from the clear solution. The insoluble acid showed m.p. 235° – 237° (d.) (preheated apparatus). The second fraction melted at 185° – 189° and after recrystallization yielded a product with m.p. 191° – 193° (preheated apparatus).

Anal. Subst. (m.p. 191° – 193°), 65.01 mg; 8.01 ml of 0.0733*N* NaOH. Equ. wt. Calc. 111.1; Found 110.7.

Subst. (m.p. 235° – 237° (d.)), 25.12 mg; CO_2 , 34.68; H_2O , 9.89 mg.

" " " 27.70 mg; 6.92 ml of 0.0721*N* NaOH.

" " " 53.42 mg; 6.54 ml of 0.0733*N* NaOH.

$\text{C}_7\text{H}_{10}\text{O}_4\text{S}_2$ (222.27) Calc. C 37.82; H 4.54; S 28.85; Equ. wt. 111.1.

Found C 37.66; H 4.42; S 28.87; Equ. wt. 111.4.

c. With iodine.—1.5 g (0.0067 mole) of *meso*- α,α' -dimercaptopimelic acid was neutralized as above (*a* and *b*) and then oxidized with iodine (iodine dissolved in an aqueous potassium iodide solution). When the colour of the reaction mixture (pale yellow) indicated excess of the oxidizing agent, a few drops of aqueous sodium thiosulphate solution was added. Purification procedure as above. 1.05 g (70.5 %) of crude product, m.p. about 180° , was obtained. One recrystallization from water gave 0.75 g (50 %) of disulphide acid with m.p. 185° – 192° . Repeated crystallization of this acid raised the melting point to 191° – 193° (preheated apparatus).

This oxidation experiment also yielded a small amount (0.05 g) of the above-mentioned high-melting, insoluble acid; m.p. 235° – 237° (d.).

Anal. Subst. (m.p. 191° – 193°), 55.10 mg; 7.15 ml of 0.0690*N* NaOH. Equ. wt. Calc. 111.1; Found 111.7.

Subst. (m.p. 235° – 237° (d.)), 27.90 mg; 3.60 ml of 0.0690*N* NaOH. Equ. wt. Calc. 111.1; Found 112.3.

The high-melting disulphide acid (m.p. 235° – 237° (d.)) has a very low solubility in water and the common organic solvents. It has a typical polymeric appearance. It is hoped to be able to continue the experiments on the molecular weight determination later on.

Racem-1,2-dithiepane-3,7-dicarboxylic acid

A. From racem- α,α' -dibromopimelic acid.—A procedure analogous to that described above for the *meso* isomer was used. Thus 15.9 g (0.050 mole) of *racem*- α,α' -dibromopimelic acid was treated with sodium disulphide. The crude product obtained (not quite solid) was rapidly dissolved in a small volume of hot water; on cooling a crystalline substance separated (1.7 g; 15 %) with m.p. 160° – 165° . Two further recrystallizations from water gave pure racemic disulphide acid (colourless needles), m.p. 171° – 173° (preheated apparatus). The fact that this acid is more soluble in water and the common organic solvents than the *meso* isomer causes greater loss of substance during the purification work.

It should be mentioned that the synthesis can also be performed with an α,α' -dibromopimelic acid, which is not stereochemically pure but richer in the racemic form. This is also obvious from the melting point diagrams (see Fig. 1 in this paper and Fig. 1 in (6)).

Anal. Subst., 27.38 mg; CO_2 , 38.00 mg; H_2O , 10.45 mg.

" " 27.49 mg; 6.91 ml of 0.0721*N* NaOH.

" " 60.64 mg; 7.56 ml of 0.0721*N* NaOH.

$\text{C}_7\text{H}_{10}\text{O}_4\text{S}_2$ (222.27) Calc. C 37.82; H 4.54; S 28.85; Equ. wt. 111.1.

Found C 37.87; H 4.27; S 29.05; Equ. wt. 111.3.

The molecular weight was determined by the ebullioscopic method with acetone as solvent (cf. 13). Mol. wt. Calc. 222.3; Found 213.

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B. By oxidation of racem- α,α' -dimercaptopimelic acid.—1.5 g (0.0067 mole) of racem- α,α' -dimercaptopimelic acid was oxidized with iodine as described in *B* (c) above for the meso acid. 1.0 g (67 %) of crude product (m.p. about 140°) was isolated. Two recrystallizations from water yielded the pure disulphide acid (0.50 g; 34 %) with m.p. 172°–173° (preheated apparatus).

No high-melting acid of polymeric nature could be found in this case.

Anal. Subst., 67.10 mg; 8.41 ml of 0.0721N NaOH. Equ. wt. Calc. 111.1; Found 110.7.

C. From meso-1,2-dithiepane-3,7-dicarboxylic acid.—4.0 g (0.018 mole) of meso-1,2-dithiepane-3,7-dicarboxylic acid was placed in a beaker and immersed in an oil bath, preheated to 199°–200°. The melting of the substance was complete after about 3 minutes and the heating was then continued for 10 minutes with stirring. After cooling, the product (m.p. 160°–170°) was pulverized and dissolved in a minimum volume of hot water, to which was added some decolourising charcoal. Upon cooling 1.9 g (47.5 %) of the meso acid, m.p. 188°–192° (preheated apparatus), was recovered. The mother liquor was evaporated and the residue fractionated by crystallizations from water. 0.50 g (12.5 %) of pure racem-1,2-dithiepane-3,7-dicarboxylic acid, m.p. 171°–173° (preheated apparatus), was obtained. The result of the rearrangement reaction can be summarized as follows: 47.5 % of the meso acid was recovered, 12.5 % of the racemic isomer was obtained and 40 % was lost by decomposition and during the fractionation procedure. This latter remark is particularly true for the racemic acid, for which the stated yield is surely too small. No separation method for the diastereomers (except fractional crystallization of the acids) could be found.

The heating should not be extended over a longer period due to decomposition of the substance.

Anal. Subst. (racemic acid), 66.52 mg; 8.30 ml of 0.0721N NaOH. Equ. wt. Calc. 111.1; Found 111.2.

Preliminary experiments on resolution.—0.22 g (0.0010 mole) of racem-1,2-dithiepane-3,7-dicarboxylic acid was dissolved in 8 ml of hot acetone. 0.47 g (0.0010 mole) of brucine (4 H₂O) was added and water dropped into the mixture, until the solution was clear (about 1 ml). Crystallization over-night in a refrigerator yielded 0.35 g of salt, from which the acid was liberated in the usual manner. The rotatory power was measured in alcohol. M.p. 162°–166°. $[\alpha]_D^{25} = +220^\circ$.

To the mother liquor from above (heated to about 50°) 0.23 g (0.0005 mole) of brucine (4 H₂O) was added together with 3 ml of water. On cooling down the solution, 0.30 g of salt deposited. The measurement of the rotatory power was performed as described above. M.p. 150°–160°. $[\alpha]_D^{25} = -382^\circ$.

Anal. Subst. ((+)-form), 38.01 mg; 4.70 ml of 0.0721N NaOH. Equ. wt. Calc. 111.1; Found 112.2. — Subst. ((-)-form), 22.77 mg; 2.82 ml of 0.0721N NaOH. Equ. wt. Calc. 111.1; Found 112.0.

From meso-1,2-dithiepane-3,7-dicarboxylic acid the following salts have been prepared:

Acid brucine salt from acetone–water.

Neutral brucine salt from acetone or water.

Acid strychnine salt from alcohol–water.

Neutral quinine salt from alcohol.

The acids liberated from these salts were all optically inactive. They showed unchanged melting points. No salts were obtained with cinchonine, cinchonidine and quinidine in different solvents.

The preparations for the *melting point diagram* were made by dissolving exactly weighed amounts of the components in acetone of high purity. The solvent was evaporated at room temperature and the residues dried *in vacuo* after powdering. The melting points were determined in capillary tubes (calibrated thermometer; electrically heated apparatus). All the determinations were made with preheated apparatus.

Summary

The diastereomers of α,α' -bis-ethylxanthogen pimelic acid, α,α' -dimercaptopimelic acid and 1,2-dithiepane-3,7-dicarboxylic acid (I) have been investigated.

Different methods for the oxidation of α,α' -dimercaptopimelic acid have been studied.

A comparison with the adipic acid series is drawn with special reference to the cyclic disulphide acids.

A melting point diagram for the system *racem*- and *meso*-1,2-dithiepane-3,7-dicarboxylic acid is given.

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The diastereomers of α,α' -dibromoglutaric acid. A note on the question of configuration

By LENNART SCHOTTE

With 1 figure in the text

The diastereomers of α,α' -dibromoglutaric acid were first described by THIELE (1). The preparation involved the oxidation of *cis*- and *trans*-1,4-dibromo-2,3-dihydroxy-cyclopentane which are intermediate oxidation products of the isomers of 1,4-dibromo-cyclopentene-2. The original intention of THIELE was to prove the attack in the 1,4-positions, when bromine is added to cyclopentadiene. The solid isomer of 1,4-dibromo-cyclopentene-2 was assigned the *trans* configuration ("unter der gewiss gerechtfertigten Voraussetzung, dass das feste die *trans*-Form, das flüssige die *cis*-Form ist"), and as a consequence the related α,α' -dibromoglutaric acid (the low-melting form) was characterized as racemic.

In 1921 INGOLD (2) prepared the two diastereomers by bromination of glutaric acid (the usual procedure with subsequent hydrolysis in formic acid) and described their separation by means of continuous chloroform extraction. The substances obtained were in good agreement with those of THIELE, and INGOLD accepted the configurations assigned by the former author. From the bromo acids INGOLD also prepared the related α,α' -dihydroxyglutaric acids and the *cis*- and *trans*-lactone acids derived from them.

The configuration problem in question has been further investigated by FARMER and SCOTT (3) and by ING and PERKIN (4). The latter authors tried to find evidence for the configurations of the diastereomers in reactions with sodium malonic ester to give different sterical isomers of cyclobutane-1,2,3-tricarboxylic acid. The reactions gave, however, only mixtures of isomers and no conclusions could be drawn. In general, the *racem* $\rightleftharpoons$ *meso* rearrangements seem to occur readily in the case of α,α' -dibromoglutaric acid and its esters (4, 5, 6).

The assignment of configuration to the diastereomers of α,α' -dibromoglutaric acid is thus based merely upon an assumption and the reactions described imply substitutions at the asymmetric centres under conditions which make sterical conclusions unreliable. It seemed therefore justified to try to investigate the problem more precisely. This intention was further strengthened by a recent result of the present author involving the formation of *racem*-1,2-dithiolane-3,5-dicarboxylic acid from the high-melting dibromo acid (assigned the *meso* configuration) and sodium disulphide (7, 8). A rearrangement during the reaction is certainly not excluded (could even be easily accounted for) but the result might also indicate that the hitherto accepted configuration of the dibromo compounds is erroneous.

Table 1. The infrared absorption of the diastereomers of α,α' -dibromoglutaric acid. (NaCl prism region; Nujol emulsion phase.)

b = broad; vs = very strong; s = strong; m = medium; w = weak; sh = shoulder;
e = emulsion peak.

High-melting form			Low-melting form		
Wavelength in μ	Wavenumber in cm^{-1}	Character of the band	Wavelength in μ	Wavenumber in cm^{-1}	Character of the band
3.47	2882	e? ¹	3.47	2882	e? ¹
5.79	1727	vs	3.8	2632	sh
6.86	1458	e	5.80	1724	vs
7.01	1427	s	5.83	1715	sh
7.27	1376	e	6.87	1456	e
7.35	1361	w	7.02	1425	s
7.85	1274	vs	7.27	1376	e
8.26	1211	s	7.74	1292	m
8.57	1167	s	7.85	1274	s
9.07	1103	w	8.14	1229	m
9.55	1047	m	8.27	1209	m
10.33	968	w	8.37	1195	m
10.81	925	m	8.59	1164	s
11.25	889	m b	9.15	1093	w
12.82	780	w	9.56	1046	m
13.20	758	w	10.42	960	w
13.90	719	e	10.82	924	w
14.50	690	m	11.17	895	m b
14.88	672	s	11.83	845	w
			13.25	755	w
			13.90	719	e
			14.50	690	m
			14.90	671	m

¹ The emulsion peak, normally found at 3.42μ , is surely influenced by an absorption band in the vicinity.

A great number of resolution experiments have been made with different alkaloids, in various solvents and under different conditions but all failed to give any information about the configurations. In some cases precipitates are formed, but they have been found to consist of either the hydrobromide of the alkaloid (characterized for brucine and morphine) or salts of lactone acids. It is obvious that the elimination of hydrogen bromide with lactone formation is too rapid to allow a resolution. It may thus be concluded that in this case methods involving ionization of the carboxylic groups cannot be used for optical resolution and establishment of configuration.

For comparison it should be mentioned that difficulties were also encountered in the resolution of α,α' -dibromosuccinic acid (9, 10), although somewhat different in character. The elimination of hydrogen bromide is here not directly connected with the ionization of the carboxylic groups and can be avoided, if the resolution is made in acid medium.

In a series of stereochemical studies on dicarboxylic acids the present author has studied the infrared absorption of some α,α' -disubstituted acids of different types (11, 12, 13, 14, 15) (in collaboration with Dr. ROSENBERG). The infrared approach to stereochemical problems in general has in fact proved promising, and certain

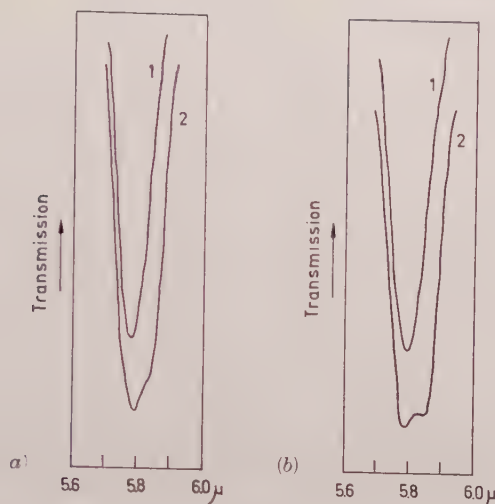


Fig. 1. IR absorption between 5.6 and 6.0 μ of high-melting (1) and low-melting (2) α, α' -dibromoglutaric acid; (a) at room temperature; (b) at -170° . CaF_2 prism, KBr phase.

regularities have been pointed out in the differences found between the spectra of meso and racemic forms of succinic and glutaric acid derivatives. Especially in the succinic acid series significant effects of different spatial distribution have been observed. The splitting of the carbonyl stretching frequency for the racemic isomer into double peaks in such cases has been regarded as the most outstanding difference in the spectra of a pair of diastereomers. It should be mentioned that the effects observed are independent of the order of the melting points of the diastereomers (15). An interpretation has been suggested for the effects (14).

By this spectral method indications about the configuration of the diastereomers of α, α' -dibromoglutaric acid are certainly obtained, but as the differences found are small, the assignment cannot be regarded as incontrovertible. When examined in the sodium chloride prism region both the acids show single peaks (the low-melting isomer, however, also exhibits a shoulder) in the carbonyl stretching region. The exact wavelengths are reported in Table 1. However, with higher resolution even small effects may be studied, and hence the wavelength region in question was also investigated with a calcium fluoride prism. Even more detailed knowledge about an absorption band is obtained, if the spectrum is recorded in a low-temperature cell (cf. 16). Care in interpretation should, however, be exercised here as small changes in the packing of the crystal lattice may be responsible for possible shifts. The carbonyl stretching frequency for the high-melting dibromo acid was found at 5.79 μ at -170° (reached with liquid air) (5.78 μ at room temperature) as a single peak, whereas the isomer shows a splitting of the band into two peaks at 5.79 μ and 5.84 μ (5.79 μ and a shoulder at 5.83 μ at room temperature). These spectral details can be seen in Fig. 1.

It should be pointed out that in the cases examined within the succinic and glutaric acid series splitting of the absorption bands in question has always been observed for the racemic isomers, even if the effects are sometimes small and only detectable under special conditions. Single peaks have been found for the meso isomers, except for α, α' -dimethylglutaric acid, where both the diastereomers show splitting of the

carbonyl stretching frequency. However, the effect seems to be of intermolecular nature for the *meso* acid.

Attempts have also been made with melting point diagrams to correlate the bromo acids to a related acid with established configuration. Combinations of *racem*- and *meso*- α,α' -dimethylglutaric acid and the diastereomers of α,α' -dibromoglutaric acid were investigated. For comparison the systems of *racem*- and *meso*- α,α' -dimethyl- and α,α' -dibromoadipic acid were also studied. The curves were all of the eutectic type; no sure indications of the existence of solid solutions have been found (decompositions near the melting points rendered the determinations somewhat difficult). Thus no conclusions as to the configuration of the diastereomers of α,α' -dibromoglutaric acid can be drawn.

Attention may be called to the fact that *meso*- α,α' -dimethylglutaric acid is the low-melting isomer (17), whereas the reverse is true for α,α' -bis-iodomethyl-glutaric acid (18). All the homologous *meso* α,α' -dibromo acids known are high-melting (cf. Table 1 in (19)). Definite evidence for α,α' -dibromoglutaric acid may probably be given by complete X-ray analysis or by electron diffraction on the isomers of 1,4-dibromo-cyclopentene-2.

Experimental

The *diastereomers of α,α' -dibromoglutaric acid* were obtained according to INGOLD (2). The high-melting acid was best recrystallized (twice) from ethylene bromide. M.p. 172°. The low-melting acid was purified in several ways after the original separation. In spite of extensive work, the melting point could not be raised above 136°, whereas INGOLD gives m.p. 142°. Examples of the purification procedure: recrystallizations three times from toluene, twice from mixtures of acetone and benzene and once from toluene, followed by extraction with chloroform and final recrystallization from toluene; recrystallization from ethylene bromide, repeated extraction with chloroform, recrystallizations from chloroform, light petroleum (b.p. 40°–60°), chloroform and ethylene bromide in the order mentioned. The acid had the appearance described by INGOLD (2) as characteristic for the pure low-melting compound ("curious, speckled appearance").

Anal. Subst. (acid m.p. 172°), 66.41 mg; 6.49 ml of 0.0709N NaOH. Equ. wt. Calc. 145.0; Found 144.3. — Subst. (acid m.p. 136°), 57.72 mg; 5.65 ml of 0.0709N NaOH. Equ. wt. Calc. 145.0; Found 144.1.

Precise determinations are impossible due to the rapid decomposition of the substances. The values given are obtained by rapid titrations and with bromothymol blue as an indicator.

The *resolution experiments* have been performed in water, methanol, ethanol, acetone, dioxane, chloroform or ethyl acetate. The alkaloids used (as free base or as salts) are: brucine, strychnine, cinchonidine, cinchonine, quinine, quinidine, morphine, *l*-ephedrine and (–)- α -phenethylamine.

For the *infrared absorption measurements* (wavelength region 2–14 μ) a Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prism was used. The substances were examined as Nujol emulsions or as solid pressed potassium bromide plates. For a closer study of the carbonyl bands a calcium fluoride prism was inserted. In addition, a low-temperature investigation was made using a technique due to ROSENBERG (16). The calibration of the absorption curves, linear in wavelength, is correct to $\pm 0.01 \mu$.

Summary

It has been established that methods involving ionization of the carboxylic groups cannot be used for the determination of the configuration of the diastereomers of α,α' -dibromoglutaric acid.

The use of melting point diagrams of mixtures with related acids has also proved unsuccessful.

From the differences observed between the IR spectra of the diastereomers (CaF₂ prism; low-temperature cell) indications are obtained, which strengthen the hitherto accepted assumption that the high-melting acid has the *meso* configuration. The effects are, however, small and cannot be regarded as incontrovertible.

ACKNOWLEDGEMENTS

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Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, January 1956.

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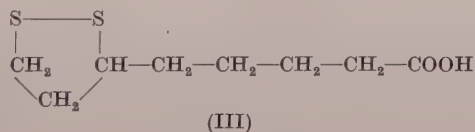
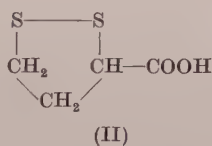
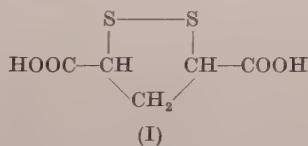
Racem-1,2-dithiolane-3,5-dicarboxylic acid and some related compounds

By LENNART SCHOTTE

With 1 figure in the text

The study of cyclic disulphide dicarboxylic acids has hitherto been performed on the adipic and pimelic acid derivatives (1, 2, 3, 4). A preliminary paper on the racemic disulphide acid related to glutaric acid (I) was presented in 1954 by the present author (5). Since then special attention has been paid to the investigation of the 1,2-dithiolane system. CLAESON has described the preparation and characteristics of 1,2-dithiolane-3-carboxylic acid (II) (6) and the present author has examined some 3-, 4- and 3,5-substituted 1,2-dithiolane derivatives from the spectrochemical point of view (7, 8, 9). The discovery of 6-thioctic acid (α -lipoic acid) (III) some years ago has moreover inspired added interest in the study of the five-membered cyclic disulphide system.

1,2-Dithiolane-3,5-dicarboxylic acid (I) can be prepared from α,α' -dibromoglutaric acid in reaction with sodium disulphide and also by the oxidation of α,α' -dimercaptoglutaric acid. As these reactions can be expected to proceed without racem $\rightleftharpoons$ meso rearrangements (2, 4, 10), it is surprising to find that the racemic disulphide acid is obtained from the high-melting form of α,α' -dibromoglutaric acid, which is ascribed the meso configuration (11, 12).

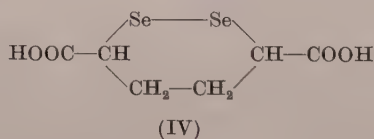


As the configuration of the diastereomers of α,α' -dibromoglutaric acid is still an open question, the present author has intensively investigated the configuration problem, but no quite definite conclusions can yet be drawn. The results will be presented separately.

The above mentioned results in the sodium disulphide reaction suggest that either the hitherto accepted configuration for the dibromo acids is erroneous or the cyclization reaction involves a mechanism, which allows steric rearrangements. It was tentatively (5) suggested that the reaction could imply an intermediate formation of a γ -lactone with subsequent attack of the disulphide group within the molecule to give the cyclic disulphide dicarboxylic acid. Such a mechanism should involve two inversions at one of the asymmetric centres and one at the other, thus resulting in a conversion from meso to racemic structure. However, studies on molecular models and recent work on the attack of sulphur-containing groups on γ -butyrolactone (13) indicate the improbability of such an interpretation. Without going any further into the problem it seems justified to conclude that if the structure of the dibromo acids is correct the conversion is in some way connected with the strained system which is formed. It should be mentioned that the yield of monomeric substance is improved at higher dilution and that an excess of sodium disulphide is favourable. The effect of dilution is, however, not at all so pronounced as has previously been reported for the seven-membered disulphide system (4). When a comparison is drawn it must, however, be remembered that the competitive reactions are different: for the 1,2-dithiolane derivative the lactone formation and for the seven-membered disulphide acid the polymerization.

The racemic disulphide acid has also been prepared by oxidation of the related dimercaptan. No difference is found with different oxidizing agents and the reaction seems to proceed more rapidly than in the pimelic acid series and to give only monomeric disulphide. In that respect the oxidation process resembles that for the adipic acid derivative.

In spite of several attempts no disulphide acid could be obtained from the low-melting form of α,α' -dibromoglutaric acid. For comparison, it is of interest to note that in the preparation of *meso*-cyclotetramethylene-diselenide-3,6-dicarboxylic acid (IV) amorphous products of polymeric nature are always obtained as by-products (14). It is very tedious work to get the monomer pure and even the pure *meso* acid shows a pronounced tendency for polymerization. The influence of steric factors on the ring closure is obvious.



The fact that it has not been possible to isolate *meso*-1,2-dithiolane-3,5-dicarboxylic acid may suggest an appreciable instability of the compound. A readily occurring rearrangement to the racemic isomer or steric conditions unfavourable for ring closure (or combinations of the factors) may also account for the observation.

The spectrochemical properties of *racem*-1,2-dithiolane-3,5-dicarboxylic acid are given in (7) and compared to the homologous acids with larger rings. The optical resolution of the acid has been accomplished. The *acid* brucine salt, crystallized from a mixture of water and ethanol, yields the (+)-acid, while the antipode is obtained from the *neutral* brucine salt. From Fig. 1 it is obvious that the two enantiomorphs form a true racemic compound with a rather small difference in melting point between the active and racemic acid. The melting point of the racemic disulphide obtained by mixing the antipodes, is in good agreement with that found in the above

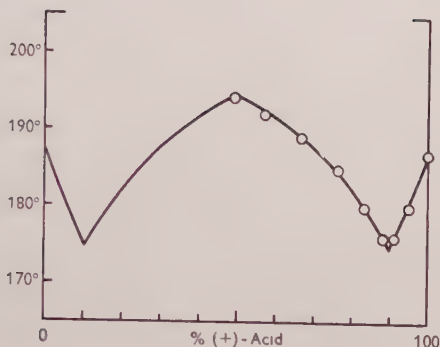


Fig. 1. Melting point diagram for the system (+)- and (-)-1,2-dithiolane-3,5-dicarboxylic acid.

mentioned sodium disulphide reaction. The acid isolated there is thus not contaminated with the meso isomer.

The enantiomorphs of 1,2-dithiolane-3,5-dicarboxylic acid show a very high molecular rotation in different solvents (abs. ethanol, $[M]_D^{25} = +1092^\circ$ and -1088° resp.); see the experimental part. It should be noticed that the rotation in aqueous solution depends strongly on the degree of dissociation. The neutral salt has an appreciably lower molecular rotation than the acid in water or 2N hydrochloric acid. This observation is surely to be connected with the gradual change of the character of the UV spectrum upon neutralization of the acid (7). The high rotatory power of the substance is probably due to the combined effects of cyclic structure, the electronic conditions of the disulphide group and the position of this group relative to the asymmetric centres. FREDGA has drawn attention to the general influence of the disulphide group on the optical rotation; an effect, which is specially pronounced when the S-S group is directly linked to the asymmetric centres (15). The values found for the enantiomorphs of 1,2-dithiolane-3,5-dicarboxylic acid also suggest a special effect of the strained five-membered disulphide ring, as the values for the corresponding 1,2-dithiane compounds are in general lower (2). Even if we consider the presence of two asymmetric centres in 1,2-dithiolane-3,5-dicarboxylic acid, we can establish that the molecular rotation is considerably higher than that of (+)- and (-)-6-thioctic acid (α -lipoic acid) (16).

A remarkable tendency to racemize has been found for the enantiomorphs of 1,2-dithiolane-3,5-dicarboxylic acid. This effect was first observed when a sample of optically active acid was recrystallized from water. Boiling of an aqueous solution for a few minutes was shown to cause racemization to about 15%. It was established by UV absorption that this decrease in activity was not connected with ring opening. It has also been proved that this racemization tendency seems characteristic for the 1,2-dithiolane derivative since the six-membered cyclic disulphide acid under analogous conditions is quite optically stable. It should be mentioned that the optical activity of the enantiomorphs of the 1,2-dithiolane-3,5-dicarboxylic acid is unchanged, if recrystallizations are made rapidly and at temperatures below 100° . No racemization was found for a solid sample after heating to 125° during 30 minutes.

The photolysis of the five-membered cyclic disulphide system has been investigated by CALVIN and co-workers (17, 18), and the present author has given some qualitative data for the racemic form of the related 3,5-dicarboxylic acid (7). The same result,

with complete disappearance of the characteristic UV absorption peak upon sufficiently long exposure, was obtained for the enantiomorphs and further evidence for the cleavage of the ring was given by the simultaneous decrease in optical activity.

From the survey of different methods for the preparation of α,α' -dimercaptodicarboxylic acids, recently published by the present author (10), it is obvious that special conditions must be applied in the preparation of α,α' -dimercaptoglutaric acid. It was found advisable to use the method involving action of alkali thiolacetate on the bromo esters and hydrolysis of the thiol esters formed. The introduction of two sulphur-containing groups into the free acids is impossible. However, the reactions between the diastereomers of dimethyl- α,α' -dibromoglutarate (prepared from the acids with diazomethane (19)) and alkali thiolacetate after hydrolysis give mixtures of *racem*- and *meso*- α,α' -dimercaptoglutaric acid. *Racem* $\rightleftharpoons$ *meso* rearrangement occurs during the hydrolysis reaction as found for the adipic acid derivative but it is possible that in the case of the glutaric acid series even the substitutions may proceed partly with conversion. Because of the hydrolysis rearrangements it is difficult to get clear information on the replacement reaction; the acid mixture obtained has been subjected to fractional crystallization but the solubility conditions made it impossible to obtain both the components sterically pure. The acid with the lowest solubility was found to possess the racemic configuration. The substitution reaction has been performed in solvents with different dielectric constants in order to try to vary the course of the substitution. Even if small effects seem to occur, the hydrolysis conversions render reliable conclusions impossible.

The enantiomorphs of α,α' -dimercaptoglutaric acid have been prepared by reduction of the antipodes of 1,2-dithiolane-3,5-dicarboxylic acid with zinc in ammoniacal solution. A great difference in molecular rotation is found between disulphide and mercaptan, and the reduction yields the mercapto acid of opposite rotation. The optical activity has been measured in different solvents (see the experimental part). The melting point of the active acids is found to be appreciably lower than that of the racemic compound. Preliminary work on the related melting point diagram reveals a close similarity to the curve given by FREDGA (20) for the antipodes of α,α' -dimethylglutaric acid. However, since great care must be exercised in the melting point determinations for the mercapto acids due to partial decomposition, probably with lactone formation, the curve has to be studied more carefully and will be presented later on. Lactone formation was also found in acid solution, which made measurements for control of optical stability (cf. above for the related disulphide) inadequate.

The mercapto groups in α,α' -dimercaptoglutaric acid may be characterized as the dibenzyl derivative. Thus the mixtures obtained in the above mentioned reactions were treated with benzyl chloride and again the racemic compound was found to be the least soluble. No complete separation of the diastereomers was possible. A mixture of the dibenzyl compounds was also obtained in reactions between the diastereomers of dimethyl α,α' -dibromoglutarate and benzylmercaptan with subsequent hydrolysis. It was surprising to find that debenzylation reactions with sodium in liquid ammonia (cf. 21, 22) were unsuccessful in spite of several experiments.

Finally it should be mentioned that current investigations of the absolute configurations of the optically active acids will be reported separately.

Experimental

Racem-1,2-dithiolane-3,5-dicarboxylic acid

A. From α,α' -dibromoglutaric acid and sodium disulphide.—14.5 g (0.050 mole) of the high-melting form of α,α' -dibromoglutaric acid (m.p. 172°) was suspended in 150 ml of water and rapidly neutralized with 8.4 g (0.10 mole) of sodium bicarbonate. An aqueous solution of sodium disulphide (0.12 mole) (prepared 48 hours before use from $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ and sulphur in water as solvent) was added immediately with cooling and the total volume adjusted to about 375 ml. The mixture was shaken occasionally and placed in a refrigerator for 10 hours and then for another 14 hours at room temperature. The alkaline solution was then acidified with dilute sulphuric acid. After the removal of precipitated non-crystalline material, mainly sulphur, the solution was extracted with ether (15 times). The ether solution was dried with anhydrous sodium sulphate. The solvent was then allowed to evaporate spontaneously and left a semi-solid mass, which became quite solid after being kept *in vacuo*. The acid was recrystallized (should be rapidly performed) from water or ethyl acetate to give beautifully formed prisms (colourless or very pale yellow). 2.1 g (22 %) of product was obtained with m.p. 191° – 195° . Another recrystallization yielded an acid with m.p. 194° – 195° .

The melting point depends on the rate of heating; the determination should be made with rapid heating or in a preheated apparatus.

Anal. Subst., 34.93 mg; CO_2 , 39.71 mg; H_2O , 10.05 mg.

„ , 11.24 mg; 6.95 ml of 0.0334*N* NaOH.

„ , 27.35 mg; 8.39 ml of 0.0334*N* NaOH.

$\text{C}_5\text{H}_6\text{O}_4\text{S}_2$ (194.22) Calc. C 30.92; H 3.12; S 33.01; Equ. wt. 97.1; Mol. wt. 194.

Found C 31.03; H 3.21; S 33.11; Equ. wt. 97.6; Mol. wt. 189.

The molecular weight has been determined by the ebullioscopic method with acetone as a solvent (cf. 8).

The racemic disulphide acid is soluble in water, alcohols, acetone, chloroform and ethyl acetate but has a very low solubility in such hydrocarbons as ligroin and benzene.

It should be mentioned that lower yields are obtained in the above reaction, if the mixture is more concentrated and no excess of sodium disulphide is used. The reaction is then accompanied by the separation of sulphur also from the alkaline medium.

Analogous experiments with the low-melting form of α,α' -dibromoglutaric acid (m.p. 136°) did not yield any crystallized disulphide acid. Several attempts under varying conditions have been performed.

B. From racem- α,α' -dimercaptoglutaric acid by oxidation.—0.4 g (0.002 mole) of racem- α,α' -dimercaptoglutaric acid was dissolved in an aqueous solution (70 ml) of sodium bicarbonate (excess) and a few drops of ferric chloride added as an indicator and catalyst. Oxygen was bubbled through the solution until the blue-violet colour had completely disappeared. Acidification with dilute sulphuric acid and extraction with ether was followed by a purification procedure as in *A*. 0.30 g (77 %) of crude product was obtained (m.p. 185° – 190°), which after recrystallization from water gave a pure disulphide acid, m.p. 193° – 195° .

Anal. Subst., 49.50 mg; 6.86 ml of 0.0746*N* NaOH. Equ. wt. Calc. 97.1; Found 96.7.

Oxidations with hydrogen peroxide and iodine resp. have given about the same yields as above.

Resolution of racem-1,2-dithiolane-3,5-dicarboxylic acid.—8.6 g (0.044 mole) of the racemic acid and 20.4 g (0.044 mole) of brucine (4 H₂O) were dissolved in a hot mixture of 220 ml of water and 135 ml of ethanol. The solution was left for crystallization at room temperature. After one day the precipitate was filtered off, dried and weighed. About 0.3 g was taken for preliminary measurement of the optical activity (on the liberated acid) and the rest recrystallized from mixtures of ethanol and water. The results are given below.

Crystallization	1	2	3	4
ml of ethanol	135	150	100	80
ml of water	220	150	100	80
Salt obtained, in g	13.1	9.8	7.7	5.8
$[\alpha]_D^{25}$ of acid (abs. ethanol)	—	+518°	+542°	+538°

10.1 g (0.022 mole) of brucine (4 H₂O) was added to the above first mother liquor (with heating to 50°–60°) and the solution was placed over-night in a refrigerator. The salt obtained yielded almost pure active acid but was recrystallized twice.

Crystallization	1	2	3
ml of ethanol	135	100	75
ml of water	220	100	75
Salt obtained, in g	19.8	14.4	11.6
$[\alpha]_D^{25}$ of acid (abs. ethanol)	–542°	–545°	–546°

It may be noted that quinine yields acid and neutral salts (in alcohol) containing excess of the levorotatory acid but the resolution is much less than that obtained with brucine ($[\alpha]_D^{25} = -128^\circ$ and -13° resp.).

(+)-1,2-Dithiolane-3,5-dicarboxylic acid

The acid brucine salt was decomposed with 4*N* sulphuric acid and the liberated acid was extracted with ether. After evaporation of the solvent (previous drying) the remaining acid was recrystallized from water (must be rapidly performed without heating above 80°) or from a mixture of ethyl acetate and ligroin. M.p. 185°–187°.

The melting point depends somewhat on the rate of heating but to a lesser extent than for the racemic acid. All melting points have been taken in the same manner in order to allow comparison.

Anal. Subst., 27.35 mg; 8.38 ml of 0.0334*N* NaOH. Equ. wt. Calc. 97.1; Found 97.7.

The solubility of the optically active acid is greater than that of the racemic in the above mentioned solvents.

The optical activity of the dextrorotatory acid was measured in different solvents (the solutions made up to 10.00 ml). The results are given below.

Solvent	mg of acid	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
2 <i>N</i> Hydrochloric acid	29.33	+4.25°	+725°	+1407°
Water	24.11	+3.38°	+701°	+1361°
Water (acid salt)	24.00	+2.75°	+573°	+1113°
Water (neutral salt)	25.56	+1.81°	+354°	+688°
Glacial acetic acid	30.46	+4.61°	+757°	+1470°
Abs. ethanol	45.88	+5.16°	+562°	+1092°
Ethyl acetate	23.07	+2.60°	+564°	+1095°
Acetone	30.61	+3.53°	+577°	+1120°

(-)-1,2-Dithiolane-3,5-dicarboxylic acid

The acid was liberated from the neutral brucine salt as described above for the enantiomorph. The levorotatory acid resembled its antipode in all respects. M.p. 185°–187°.

Anal. Subst., 30.53 mg; 9.40 ml of 0.0334*N* NaOH. Equ. wt. Calc. 97.1; Found 97.2.

33.11 mg of the acid in 10.00 ml of abs. ethanol; $2\alpha_D^{25} = -3.71^\circ$; $[\alpha]_D^{25} = -560^\circ$; $[M]_D^{25} = -1088^\circ$.

Racemization of optically active 1,2-dithiolane-3,5-dicarboxylic acid

48.50 mg of the (+)-acid ($[\alpha]_D^{25} = +701^\circ$ (in water)) was dissolved in 10 ml of water and the solution boiled for 5 minutes. The specific rotation was thereby lowered to $+603^\circ$. Under analogous conditions (–)-1,2-dithiane-3,6-dicarboxylic acid was quite stable.

An aqueous solution (10 ml) of 23.27 mg of levorotatory acid (not quite optically pure) with $[\alpha]_D^{25} = -662^\circ$ was maintained at 72.5° for 12.5 hours. A decrease in specific rotation to -627° was found. A further decrease (to -574°) was obtained after boiling this solution for 4 minutes.

When a sample of the (+)-acid was heated to 125° for 30 minutes no change was observed in the specific rotation.

An alcoholic solution (10 ml) of 26.00 mg of (–)-1,2-dithiolane-3,5-dicarboxylic acid was photolyzed at room temperature and the specific rotation measured after various time intervals.

Time in minutes	0	10	30	90	130
$[\alpha]_D^{25}$	-561°	-465°	-337°	-127°	-125°

Racem- α,α' -dimercaptoglutaric acid

1.3 g (0.0067 mole) of *racem*-1,2-dithiolane-3,5-dicarboxylic acid was dissolved in 15 ml of 4 *N* ammonia and the solution sucked through a layer of zinc powder four times. The reaction mixture was then neutralized with dilute sulphuric acid and the precipitated zinc mercaptide filtered. The acid was liberated and the dimercapto acid extracted with ether from the aqueous solution. 1.1 g (85 %) of crude product (m.p. 130°–134°) was isolated and yielded after recrystallization from ethyl acetate 0.65 g (50 %) of pure substance, m.p. 135°–136°. Due to decomposition (probably with lactone formation) the melting point depends on the rate of heating (cf. m.p. 141° with instantaneous melting). The values given are obtained with a temperature rise of 2°/min.

Anal. Subst., 30.27 mg; CO₂, 33.92 mg; H₂O, 11.10 mg.

„ , 30.37 mg; 8.72 ml of 0.0709*N* NaOH.

„ , 43.18 mg; 6.39 ml of 0.0685*N* NaOH.

„ , 39.21 mg; 8.44 ml of 0.0475*N* J₂.

C₅H₈O₄S₂ (196.23) Calc. C 30.60; H 4.11; S 32.68; Equ. wt. 98.1.

Found C 30.59; H 4.10; S 32.63; Equ. wt. 98.6; 97.8.

The dimercapto acid is soluble in common organic solvents.

(+)- α,α' -Dimercaptoglutaric acid

0.30 g (0.0015 mole) of (–)-1,2-dithiolane-3,5-dicarboxylic acid was reduced as described above. 0.27 g (90 %) of the dextrorotatory dimercapto acid was obtained, m.p. 84°–86° (with instantaneous melting m.p. 91°).

Anal. Subst., 48.62 mg; 4.99 ml of 0.0999N J₂. Equ. wt. Calc. 98.1; Found 97.5.

54.62 mg of the acid in 10.00 ml of abs. ethanol: $2\alpha_D^{25} = +0.79^\circ$; $[\alpha]_D^{25} = +72^\circ$; $[M]_D^{25} = +142^\circ$.

(–)- α,α' -Dimercaptoglutaric acid

0.45 g (0.0023 mole) of (+)-1,2-dithiolane-3,5-dicarboxylic acid was treated as above. 0.40 g (89 %) of the levorotatory dimercapto acid was isolated, m.p. 84°–86°.

Anal. Subst., 41.55 mg; 4.25 ml of 0.0999N J₂. Equ. wt. Calc. 98.1; Found 97.9.

The optically active acids show greater solubility than the racemic acid in the common organic solvents.

The optical activity of the (–)-acid was measured in different solvents (10.00 ml of solution). The results are given below.

Solvent	mg of acid	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Water (both carboxylic groups neutralized)	62.06	–0.38°	–31°	–60°
Glacial acetic acid	41.84	–0.49°	–59°	–115°
Abs. ethanol	45.65	–0.665°	–73°	–143°
Chloroform	54.80	–0.82°	–75°	–147°
Ethyl acetate	47.89	–0.79°	–83°	–162°

Measurements in water and dilute hydrochloric acid do not give reliable results due to lactone formation. It was found that boiling of an aqueous solution (53.19 mg of the (+)-acid in 10 ml of solvent) for 5 minutes caused decomposition with the liberation of hydrogen sulphide and complete loss of optical activity.

The melting point given above for *racem- α,α' -dimercaptoglutaric acid* has been verified by the value obtained for the mixture of equal amounts of the enanthiomorphs.

 α,α' -Dimercaptoglutaric acid (mixture of the diastereomers)

18 g (0.062 mole) of high-melting α,α' -dibromoglutaric acid was esterified with diazomethane and the ester dissolved in 100 ml of absolute methanol. 10.4 g (0.14 mole) of thiolacetic acid was neutralized with 7.7 g (0.14 mole) of potassium hydroxide, dissolved in 100 ml of absolute methanol, and the solution added to the bromo ester. The separation of KBr began after about 5 minutes. The mixture was placed at room temperature over-night; most of the methanol was then removed at reduced pressure (nitrogen atmosphere!) and the residue poured into water. The ester was extracted with ether and directly hydrolyzed after removal of the solvent. For the hydrolysis, the ester was dissolved in 40 ml of absolute methanol and an aqueous solution (100 ml) of 11 g (0.2 mole) of KOH was added. The temperature was kept at about 40° and nitrogen bubbled continuously through the solution (important!). After 4 hours the mixture was cooled, acidified and extracted with ether. The ether solution was dried with anhydrous sodium sulphate, after which the solvent was distilled at reduced pressure (nitrogen). The residual α,α' -dimercaptoglutaric acid

was contaminated with acetic acid (from the hydrolysis), which could easily be removed *in vacuo*. Recrystallizations were made from ethyl acetate. Result:

10 g (82 %) of crude product, m.p. 106°–110°.

Recrystallization 1: 4.8 g (39 %), m.p. 111°–113.5°.

„ 2: 2.3 g (19 %), m.p. 116°–117.5°.

A small amount was recrystallized once more and showed m.p. 125.5°–126.5°. Mixed m.p. of this acid (3 parts) and the pure racemic acid (1 part) gave m.p. 128°–130°.

Anal. Subst., 30.45 mg: 8.70 ml of 0.0709*N* NaOH.

„ , 38.58 mg: 5.71 ml of 0.0685*N* NaOH.

„ , 33.34 mg: 7.00 ml of 0.0475*N* J₂.

C₃H₃O₄S₂ (196.23) Calc. S 32.68; Equ. wt. 98.1.

Found S 32.47; Equ. wt. 98.6; 100.3.

When the same reaction was performed with low-melting α,α' -dibromoglutaric acid (m.p. 136°) (16 g; 0.055 mole) the following result was obtained:

8.5 g (79 %) of crude product, m.p. 105°–110°.

Recrystallization 1: 4.6 g (43 %), m.p. 114°–117.5°.

„ 2: 2.8 g (26 %), m.p. 118°–120°.

Oxidation of the mixtures (recrystallized twice) yielded crude disulphide products with m.p. 150°–170° (yield: 79 % and 75 % resp.). Recrystallizations from water gave the pure racemic disulphide. The low melting point may indicate a contamination of the meso isomer, but this acid could not be isolated from the mother liquor.

Treatment with benzyl chloride (see below) gave α,α' -dibenzylmercapto-glutaric acids with m.p. 110°–118° and 100°–114° resp. Recrystallizations did not cause any separation of the diastereomers; after one crystallization the values 119°–120° and 110°–118° were obtained.

Anal. (dibenzyl derivative, m.p. 119°–120°),

Subst., 40.90 mg: 6.12 ml of 0.0709*N* NaOH.

„ , 94.78 mg: 7.34 ml of 0.0685*N* NaOH.

C₁₉H₂₆O₄S₂ (376.47) Calc. S 17.03; Equ. wt. 188.2.

Found S 17.01; Equ. wt. 188.5.

The conditions for the above thiolacetate reaction have been varied somewhat, and the results are summarized below.

a) Water-free medium with absolute methanol as solvent was used (the salt prepared from sodium and thiolacetic acid). 10 g (0.035 mole) of high-melting α,α' -dibromoglutaric acid was esterified with diazomethane and treated as above but the reaction time was extended to 3 days. Result:

Crude product: 3.5 g (51 %), m.p. 110°–115°.

Recrystallization 1: 1.2 g (17 %), m.p. 117°–121°.

„ 2: 0.7 g (10 %), m.p. 122°–125°.

The dibenzyl derivative (nearly quantitative yield) showed m.p. 115°–135°. Recrystallized once from formic acid the product melted at 118°–140°.

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b) The same procedure as in (a) with 10 g (0.035 mole) of the low-melting bromo acid.

Crude product: 3.4 g (49 %), m.p. 112°–117°.

Recrystallization 1: 1.2 g (17 %), m.p. 119°–123°.

„ 2: 0.7 g (10 %), m.p. 124°–126°.

The dibenzyl derivative had m.p. 117°–119° (88 % yield) and was recrystallized once from formic acid to give a sample with m.p. 117°–140°.

c) The procedure was analogous to that in (a) but with large excess of sodium thiolacetate.

Crude product: 4.0 g (58 %), not quite solid.

Recrystallization 1: 1.8 g (26 %), m.p. 120°–124°.

„ 2: 0.6 g (9 %), m.p. 125°–128°.

The dibenzyl derivative of the mercapto acid (m.p. 120°–124°) melted at 115°–130° and after one recrystallization from formic acid at 131°–138°. The corresponding values for the derivative of the mercapto acid with m.p. 125°–128° were 137°–142° and 148°–150° respectively.

d) 8.0 g (0.028 mole) of high-melting α,α' -dibromoglutaric acid was treated as in (a) but with isopropyl alcohol as a solvent (125 ml). The crude mercapto acid obtained was not quite solid but was recrystallized directly from ethyl acetate. The following data were found:

Recrystallization 1: 1.7 g (31 %), m.p. 111°–118°.

„ 2: 1.0 g (18 %), m.p. 124°–128°.

„ 3: 0.25 g (5 %), m.p. 131°–133°.

The dibenzyl derivative of the last compound melted at 141°–144° and after one recrystallization from formic acid at 149°–150° (cf. the racemic acid below).

It has been proved throughout by iodine titrations that the equivalent weights of the dimercapto acids are correct. It is obvious from the results that only mixtures are obtained, from which in a few cases the racemic acid could be isolated and identified with authentic samples.

It should be noted that the melting points given for the dimercapto acids in this part all refer to a temperature rise of 2°/min. The different values thus allow comparisons to be drawn.

Racem- α,α' -dibenzylmercapto-glutaric acid

0.70 g (0.0036 mole) of *racem- α,α' -dimercaptoglutaric acid* was completely neutralized with potassium hydroxide (0.95 g of 86 % sample) in 20 ml of water. An alcoholic solution (20 ml) of 0.91 g (0.0072 mole) of benzyl chloride was added and the mixture kept over-night at room temperature. After evaporation of the alcohol the residue was acidified with dilute sulphuric acid. The crude product immediately separated and was filtered off when quite crystalline. 1.3 g (96 %) of *racem- α,α' -dibenzylmercapto-glutaric acid*, m.p. 146°–150°, was obtained, which after recrystallization from formic acid yielded colourless needles with m.p. 149.5°–151°.

Anal. Subst., 24.02 mg; CO₂, 53.15 mg; H₂O, 12.00 mg.

„ , 40.02 mg; 6.01 ml of 0.0709N NaOH.

„ , 72.91 mg; 5.69 ml of 0.0685N NaOH.

C₁₉H₂₀O₄S₂ (376.47) Calc. C 60.61; H 5.36; S 17.03; Equ. wt. 188.2.

Found C 60.37; H 5.58; S 17.07; Equ. wt. 187.1.

The above mentioned derivatives of the different mixtures of the diastereomers of *α,α' -dimercaptoglutaric acid* were prepared in the same manner.

Reactions between dimethyl α,α' -dibromoglutarate and benzyl mercaptan

4.7 g (0.016 mole) of high-melting α,α' -dibromoglutaric acid was esterified with diazomethane and the ester dissolved in 50 ml of abs. methanol. A solution of sodium benzyl mercaptide in methanol was prepared from 1.1 g (0.048 mole) of sodium and 6.1 g (0.049 mole) of benzyl mercaptan in 30 ml of methanol. The ester solution was added (considerable heat was evolved) and the mixture was left at room temperature for one day. The solvent was removed at reduced pressure. Hydrolysis of the ester was not possible in acid media but was performed with potassium hydroxide (5 g) dissolved in a mixture of 50 ml of ethanol and 3 ml of water at 60°–70° for 3 hours. The alcohol was then distilled at reduced pressure until the separation of crystals began. The precipitated potassium salt was filtered off and the acid liberated. Another sample of α,α' -dibenzylmercapto-glutaric acid was obtained upon acidification of the filtrate. The following products were isolated:

From the potassium salt: Crude product, 1.6 g (27 %), m.p. 116°–119°.

Recrystallization 1, 0.8 g (13 %), m.p. 117°–119°.

From the mother liquor: Crude product, 1.5 g (25 %), m.p. 125°–134°.

Recrystallization 1, 1.2 g (20 %), m.p. 125°–140°.

Anal. Subst., 79.23 mg: 5.97 ml of 0.0709N NaOH. Equ. wt. Calc. 188.2; Found 187.2.

An analogous reaction with 4.1 g (0.014 mole) of low-melting α,α' -dibromoglutaric acid gave the following results:

From the potassium salt: Crude product, 1.2 g (23 %), m.p. 115°–120°.

Recrystallization 1, 0.8 g (15 %), m.p. 120°–125°.

From the mother liquor: Crude product, 1.3 g (25 %), m.p. 133°–141°.

Recrystallization 1, 0.9 g (17 %), m.p. 138°–145°.

It is obvious that mixtures are obtained with partial enrichment of the racemic isomer in the recrystallizations.

The preparations for the *melting point diagram* were made by dissolving exactly weighed amounts of the components in acetone of high purity. The solvent was evaporated at room temperature and the residue dried *in vacuo* after powdering. The melting points were determined in capillary tubes with preheated apparatus or rapid heating (calibrated thermometer; electrically heated apparatus except for the determinations with instantaneous melting, when a sulphuric acid bath (of the usual type) was used.

The *photolysis* of the optically active 1,2-dithiolane-3,5-dicarboxylic acid was performed with a high pressure mercury quartz lamp (Philips). The solutions were kept in a 25 ml quartz flask at a distance from the lamp of about 10 cm. The power of the ultraviolet radiation source is 125 watt and it gives a linear as well as continuous spectrum in the wavelength region under question.

Summary

Racem-1,2-dithiolane-3,5-dicarboxylic acid and some related compounds have been investigated.

The optical resolution of the disulphide acid is described and special attention is paid to the high molecular rotation (abs. ethanol, $[M]_D^{25} = +1092^\circ$ and -1088° resp.) and the racemization tendency of the enantiomorphs.

No *meso* acid could be isolated.

The replacement reaction between dimethyl α, α' -dibromoglutarate and sulphur-containing groups has been studied.

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Studies on sulphur compounds related to glutaric and pimelic acid with special reference to the 1,2-dithiolane system

By LENNART SCHOTTE

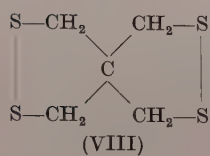
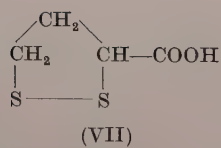
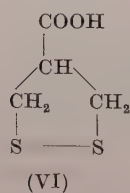
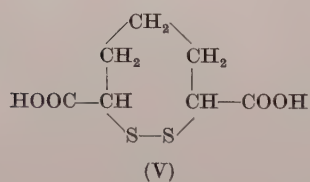
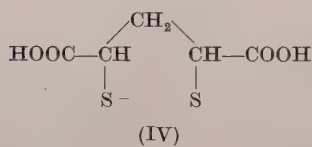
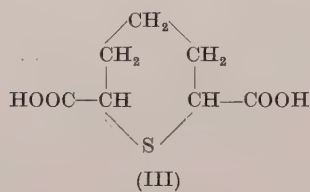
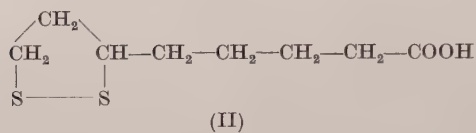
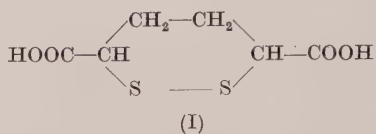
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Introduction

A thorough study of sulphur compounds related to dicarboxylic acids was first performed by FREDGA, who examined the adipic acid series in the years 1937–1938 (12–14). Thus the diastereomers of α,α' -dimercaptoadipic acid and their cyclic oxidation products, *racem*- and *meso*-1,2-dithiane-3,6-dicarboxylic acid (I), were characterized (13, 14). The cyclic disulphides are of special interest since valuable information is obtained about the properties of the S–S group. As the compounds in question contain two similar asymmetric centres, the influence of steric effects may also be studied. Supplementary information may be obtained by comparison with the corresponding selenium compounds (15–18).

The purpose of this and previously reported investigations (1–11) has been to pursue the work outlined above and extend it to glutaric and pimelic acid. It was soon found that the related disulphides were most interesting and in particular those with five-membered rings, derived from α,α' -dimercaptoglutaric acid. Special study has therefore been devoted to this 1,2-dithiolane (1,2-dithiacyclopentane) system. Difficulties, which were encountered in the preparations, have, however, necessitated some digressive studies. The properties of the cyclic disulphides have been elucidated mainly by physico-chemical methods, which include a spectrochemical investigation on disulphides in general.

The expectations of FREDGA (14) as to the importance of a closer study of cyclic disulphides have been fully realized by the discovery and synthesis of 6-thioctic acid (6,8-dithio-*n*-octanoic acid or α -lipoic acid) (II) which contains the 1,2-dithiolane ring (19–28). It has been found that this substance is active in the oxidative decarboxylation of pyruvate to active acetyl groups (29–33). CALVIN and co-workers have furthermore advanced a hypothesis that the disulphide acid itself is involved in the conversion of the electromagnetic energy of quanta into chemical bond energy (34–36). The available energy of the photochemically excited chlorophyll molecule should in such a case start photosynthesis by splitting the disulphide bond into a biradical. As this would be possible only with a sufficiently high strain energy in the 1,2-dithiolane part of the 6-thioctic acid molecule, it has become a matter of importance to study the chemistry and physics of the 1,2-



dithiolane derivatives. The reactivity of the system is directly connected with the geometry of the S-S linkage, which will be discussed later on. It should be noted here that CALVIN and his co-workers tried to calculate the strain from spectroscopic studies, from purely structural considerations together with some measured single bond rotational barriers (conformation analysis), and from kinetic studies of the equilibrium between benzyl mercaptan and 1,2-dithiolane. The results have been discussed by SUNNER (37, 38) and compared to his own, obtained in a more direct way by calorimetric experiments. SUNNER found a lower value for the strain energy of 1,2-dithiolane than those reported by CALVIN. The method involving spectroscopic studies has been particularly criticized by SUNNER, who arrives at the conclusion that the hypothesis of CALVIN about the second step of the photosynthesis cannot be correct, in any case in its original version (38). As the reference cited (38) contains a valuable review of the chemistry and energetics of 6-thioctic acid no further discussion of the matter will be given here. The problem cannot be regarded as definitely settled until the chemistry of the 1,2-dithiolane ring is known in detail.

Discussion of methods of preparation

The α, α' -dibromo-dicarboxylic acids are in general starting materials for the synthesis of related mercaptans, sulphides and disulphides. It is thus essential to know the configuration of the diastereomers to be used. In the case of α, α' -dibromoglutaric acid the assignments given (39, 40) are based only upon assumptions and so a closer investigation seemed justified (1). It was found that methods involving ionization of the carboxylic groups, e.g. resolution of the presumptive racemic acid, cannot be used for the establishment of configuration because of the rapid elimination of hydrogen bromide with lactone formation. The diastereomers were then examined as to their infrared absorption in the sodium chloride prism region in an attempt to correlate the results to recently established relationships between configuration and infrared absorption (41-45). The infrared approach to stereochemical problems in general has proved promising and especially in the succinic acid series significant effects of different spatial distribution have been observed. The results obtained from this spectral method certainly strengthen the hitherto accepted assumption about the configuration of the diastereomers of α, α' -dibromoglutaric acid, but the effects are small and should not be regarded as incontrovertible (1). Only one of the corresponding α, α' -dichloro acids is hitherto known (46).

A method of separation has been described for the diastereomers of α, α' -dibromopimelic acid (2). Conclusive evidence as to the configurations has been presented by the optical resolution of the low-melting acid. It has also been possible to characterize the enantiomorphs (2).

Although it is somewhat beyond the scope of this subject, it may be justified to call attention to the tables in reference (2), which illustrate the melting points of the diastereomers of homologous α, α' -dimethyl- and α, α' -dibromo-dicarboxylic acids (2: Table 1) and the optical activities of some enantiomorphs within the series of α, α' -dibromo acids (2: Table 2). It should be noted that in general the meso isomers are the high-melting acids but that deviation from the apparent regularity is found for α, α' -dimethylglutaric acid. The melting point curve of the antipodes of this acid, however, reveals the existence of a racemic compound of great stability, melting about 60° higher than the active forms (the meso acid also has a higher melting

point than the active acids). It may be concluded that a comparison between the melting points of meso and active acids should be more significant.

A survey of different methods for the preparation of α, α' -dimercapto-dicarboxylic acids is given in reference (3). The pimelic acid derivatives are obtained by hydrolysis of the corresponding ethyl xanthates (4) whereas the method involving intermediate formation of thioesters must be used in the case of α, α' -dimercaptoglutaric acid (3, 5). The complications in the glutaric acid series are discussed in reference (5) and will also be considered briefly below. It may be mentioned that unsuccessful attempts have been made to prepare 1,3-bis-ethylxanthogen-propane-tetracarboxylic acid-1,1,3,3 which should be easily decarboxylated to the glutaric acid derivative. Xanthogen substituted malonic acids are known to be rather unstable (47).

The sulphides related to the diastereomers of α, α' -dibromopimelic acid, *cis*-(*meso*)- and *trans*-(*racem*)-tetrahydrothiapyran-2,6-dicarboxylic acid (III), have been prepared in reactions with sodium sulphide (6). These replacements proceed without $\text{racem} \rightleftharpoons \text{meso}$ rearrangements.

The disulphides, *racem*-1,2-dithiolane-3,5-dicarboxylic acid (IV) and the diastereomers of 1,2-dithiepane-3,7-dicarboxylic acid (1,2-dithiacycloheptane-3,7-dicarboxylic acid) (V) have been synthesized from the related dibromo acids and sodium disulphide or by oxidation of the corresponding dimercapto acids (3-5, 48, 49). The oxidizing agents used are hydrogen peroxide, oxygen and iodine. For the pimelic acid series iodine has proved to be the best reagent (4), whereas no differences were found for the oxidations of *racem*- α, α' -dimercaptoglutaric acid (5). A hypothetical method for synthesis of the 1,2-dithiolane derivatives from diethyl bromo malonate and potassium disulphide with subsequent ring closure by means of formaldehyde, followed by hydrolysis and decarboxylation could be excluded due to the fact that alkali disulphides rapidly reduce diethyl bromo malonate to diethyl malonate (cf. 47).

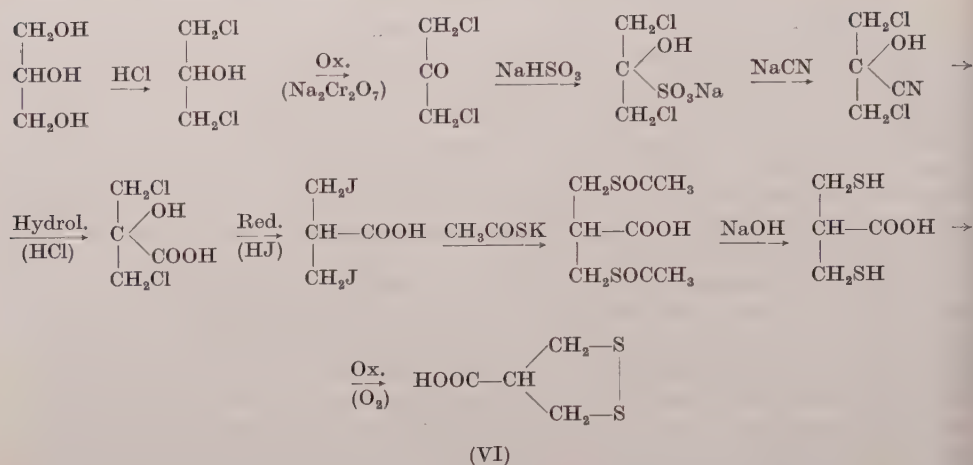
There are some remarks which should be made in connection with the methods of preparation reported above. In accordance with the observations of FREDGA as regards the alkali disulphide (12) and in particular the diselenide (15) reactions with α, α' -dibromoadipic acid it has been found that in the above mentioned synthesis of (V) some monosulphide acid (III) is also formed. Thus, both the meso acids were isolated (4, 49) and the same kind of reaction is likely also for the racemic series, where the solubility conditions, however, have rendered a separation difficult. If this result is connected with the fact that during syntheses of 1,2-dithiepane derivatives, polymeric substances are always by-products (the amounts can be reduced at high dilution), the nature of the replacements in the cyclization reactions may be rather well interpreted. No $\text{racem} \rightleftharpoons \text{meso}$ conversions are observed and thus it seems justified to conclude that after the first replacement at one of the asymmetric centres the reaction may proceed either with ring closure to monomer disulphide, with ring closure and separation of sulphur to monosulphide or by forming disulphides of polymeric character. It should be pointed out that no stable intermediate with coordinately linked sulphur has been observed. Reasons for the improbability of branched sulphur chains are given in reference (7) (see also below). The correlation between the above mentioned modes of reaction and the steric conditions is well illustrated by the fact that the two ring closure alternatives occur in the adipic acid series, where the related disulphide is six- and the sulphide five-membered. All the reaction possibilities are observed for the pimelic acid derivatives, where the disulphide ring is larger (seven-membered).

The above considerations for the disulphide reactions are not true for the glutaric

acid system. In fact, steric conversions seem to occur readily for the sulphur derivatives (5). It is found that the high-melting form of α, α' -dibromoglutaric acid (ascribed the meso configuration) gives the racemic disulphide (5, 48). If the accepted configuration is correct (see above), the cyclization reaction in question must involve a mechanism which allows steric rearrangements. As a pronounced tendency to lactone formation has been established for α, α' -dibromoglutaric acid (3), it was tentatively suggested (48) that the reaction could imply an intermediate formation of a γ -lactone with subsequent attack of the disulphide group within the molecule to give the cyclic disulphide dicarboxylic acid. Such a mechanism should involve two inversions at one of the asymmetric centres and one at the other, thus resulting in a conversion from meso to racemic structure. However, studies on molecular models and recent work by the present author on the attack by sulphur-containing groups on γ -butyrolactone (8) indicate the improbability of such an interpretation. It is certainly known from the difference in lactone stability between γ -butyrolactone- γ -carboxylic acid and γ -butyrolactone- β -carboxylic acid (50) that carboxy substitution in the γ -position considerably favours ring opening but nevertheless the steric arrangements and the reaction conditions seem inconsistent with the assumption of a lactone intermediate. The conversion can surely be best accounted for by assuming the replacements to proceed according to S_1 and S_2 mechanisms with preliminary retention and then inversion of configuration. As the reactions under discussion for the adipic and pimelic acid obviously involve S_2 substitutions only (see above), the question arises whether the observed effect is characteristic for the glutaric acid system or should be connected with the strained disulphide ring to be formed. No unambiguous answer can be given, but attention is called to the intense investigation of the reaction between the diastereomers of dimethyl α, α' -dibromoglutarate and alkali thiolacetate (5). Racem $\rightleftharpoons$ meso rearrangements during the hydrolysis render reliable conclusions about the substitutions impossible. The fact that it has not been possible to isolate any meso-1,2-dithiolane-3,5-dicarboxylic acid (see below) makes the interpretation of the reaction mechanism rather difficult (5). Regardless of the nature of the mechanism it should be remembered that the competitive reactions in the disulphide preparations are different for the glutaric and pimelic acids: for glutaric acid lactonization, for pimelic acid formation of monosulphide and polymers.

With regard to the discussion in reference (8) about the different modes of attack of mercaptides and alkoxides on γ -butyrolactone it should be mentioned that REPPE has described some reactions of γ -butyrolactone in a recent paper (51). He states that alkoxides cause ring cleavage at the alkyl carbon-oxygen bond in conformity with the results of FITTIG and STRÖM in 1892 (52). The work of the latter authors has, however, been doubted by SPENCER and WRIGHT (53), who arrived at the opposite conclusion (acyl carbon-oxygen bond fission) after a very careful investigation under proper conditions. REPPE does not seem to have been aware of this work. The assumption of the present author in reference (8) that both acyl and alkyl attack occur simultaneously but with different rate should perhaps be stressed. However, the reaction products of REPPE might be the result of secondary reactions under the conditions employed.

Finally it may be mentioned that the 4-substituted 1,2-dithiolanes, the spectrochemical investigation of which is reported in reference (9), were prepared according to BACKER and co-workers (54-56). 1,2-Dithiolane-4-carboxylic acid (VI), possibly the disulphide existing in asparagus (cf. JANSEN (57)), has been synthesized according to the following scheme (58)



Dimercapto acids

It should be pointed out that α,α' -dimercaptosuccinic acid cannot be prepared by the methods discussed above. OWEN and SULTANBAWA (59) have, however, described the preparation of a mixture of the diastereomers by addition of thiolacetic acid to acetylenedicarboxylic acid and subsequent acid hydrolysis of the bis-acetyl compound formed. No separation of the isomers was performed. Special attention may be called to the formation of α -xanthogen- γ -butyrolactone- γ -carboxylic acid in reactions between α,α' -dibromoglutaric acid and potassium ethyl xanthate, as interesting effects on the infrared absorption are found for the acid salt in particular, for the lactone acid and the neutral salt (3). The homologous α,α' -dimercaptodicarboxylic acids are collected in Table 1.

The enantiomorphs of α,α' -dimercaptopimelic acid have not yet been prepared. It should be noted that the racemic acid is the high-melting isomer, whereas the reverse is true for the related dibromo (2) and cyclic disulphide derivatives (4). α,α' -Dimercaptoglutaric acid is found to decompose readily upon heating with the formation of lactone or/and thiolactone. An account of the reactions of some thiolactones is given by SCHJÄNBERG (60).

Table 1. Homologous α,α' -dimercapto-dicarboxylic acids.

Derivative of	M.p.			Ref.
	Meso form	Racem form	Enantiomorphs	
Glutaric acid	—	135° —136°(141°)	84° — 86°(91°)	(5)
Adipic acid	188°	111.5°—112.5°	119.5°—120.5°	(14)
Pimelic acid	81°—83°	106° —107°	—	(4)

Sulphide acids

No investigation of the glutaric acid series has been made in this respect due to the properties which can be postulated for the four-membered cyclic sulphide acids on the basis of other experiments. The adipic and pimelic acid derivatives are compared in Table 2.

Table 2. Some cyclic sulphide dicarboxylic acids.

Substance	M.p.			Ref.
	Meso (<i>cis</i>) form	Racem (<i>trans</i>) form	Enanthiomorphs	
Thiophane-2,5-dicarboxylic acid	144°–145°	165°–166°	179°–180°	(12)
Tetrahydrothiapyran-2,6-dicarboxylic acid	210°–211°	148°–149°	—	(6)

The characteristics and reactions of the diastereomers of tetrahydrothiapyran-2,6-dicarboxylic acid are described in reference (6). The preliminary optical resolution of the racemic isomer has not been further pursued. It is of interest to note that steric conversions occur upon hydrolysis of the pure esters of the diastereomers, whereas the cyclization reaction proceeds without racem $\rightleftharpoons$ meso rearrangements for acid as well as ester (3). The same is true when the thiophane derivatives are formed from the diastereomers of α, α' -dibromoadipic acid (or the dimethyl esters) and sodium hydrosulphide (3); this reaction involves rapid alkylation of a mercapto group with ring closure (cf. also 61).

Disulphide acids

The cyclic disulphide acids related to glutaric, adipic and pimelic acid are collected in Table 3.

Two mono-carboxylic acid 1,2-dithiolane derivatives are also known. 1,2-Dithiolane-3-carboxylic acid (VII), m.p. 81°–82° (147°), has been described by CLAESON (62); 1,2-dithiolane-4-carboxylic acid (VI), m.p. 76.5°–77.5°, has recently been prepared by the present author and STRÖM (58) (see above).

Table 3. Some cyclic disulphide dicarboxylic acids.

Substance	M.p.			Ref.
	Meso form	Racem form	Enanthiomorphs	
1,2-Dithiolane-3,5-dicarboxylic acid	—	194°–195°	185°–187°	(5)
1,2-Dithiane-3,6-dicarboxylic acid	199°	c. 270° (d.)	c. 257°	(14)
1,2-Dithiepane-3,7-dicarboxylic acid	191°–193°	171°–173°	—	(4)

In spite of several attempts it has not been possible to prepare *meso*-1,2-dithiolane-3,5-dicarboxylic acid. This may suggest an appreciable instability of the compound but it is also likely that the steric conditions are unfavourable for ring closure. A readily occurring rearrangement to the racemic isomer or combinations of the different factors may account for the experimental result. The first two suggestions seem consistent with the observations of FREDGA (17, 18) as regards the preparation of *meso*-1,2-diselenane-3,6-dicarboxylic acid (*meso*-cyclotetramethylene-diselenide-3,6-dicarboxylic acid). Amorphous products of a polymeric nature have been found as by-products; it is very difficult to get the monomer pure and even the pure *meso* acid shows a pronounced tendency for polymerization. In this respect attention is called to the fact that a definite polymeric disulphide acid related to *meso*-1,2-dithiepane-3,7-dicarboxylic acid was isolated by the present author under certain conditions (4). A further study of this polymer could perhaps give information about the behaviour of larger disulphide rings. The disulphide related to suberic acid contains an eight-membered ring but preliminary work by FREDGA has given only polymeric products (63).

The resolution of racem-(V) has not yet been completed due to lack of substances. The preliminary tests have, however, indicated rather high molecular rotation for the enantiomorphs (4).

1,2-Dithiolane-4-carboxylic acid (VI) is of a special interest, as the related dimercaptan has been isolated from asparagus (57). JANSEN qualitatively showed the presence of disulphide and mercaptan in asparagus juice but attempts at isolation gave only polymeric disulphide products. Reduction of the polymer gave a dimercaptan which was characterized as β,β' -dimercapto-*iso*-butyric acid. These results might suggest that the disulphide present in asparagus is really of polymeric character, but the experiences of the present author in the preparation of (VI) clearly shows that not even the conditions used by JANSEN were mild enough to ensure the isolation of a possible monomer. The 1,2-dithiolane system is reactive and cleavage of the S-S bond with formation of linear polymers readily occurs. As a consequence it seems reasonable to assume the presence of (VI) in asparagus and it will be examined later to find out if the occurrence of the reactive ring system is important for the biochemical processes in the rapidly growing asparagus.

Properties of disulphides

General remarks

For a complete understanding of many of the disulphide properties it is essential to know the steric arrangement of the bonds around the S-S group. The *s* electron pair is spherically distributed about the nucleus and the *p* pairs are on the 90° axes. Thus the distribution leads to a very interesting spatial configuration for disulphides. There are two symmetrical energy minima for rotation around an S-S linkage and the dihedral (azimuthal) angle is between 90° and 100° (the angle between the two S-S-C planes). The rotational barrier corresponds to at least 10 kcal. as determined by careful spectroscopic studies on dimethyl disulphide (64) and diethyl disulphide (65). Energy maxima are found for the *trans*- and *cis*-position, of which the latter with a dihedral angle of 0° may from steric reasons be expected to have somewhat higher energy (66). For 2,3,4-trithia-*n*-pentane (67) a dihedral angle of 93° is reported, whereas PAULING found 102° for the ordinary S₈ molecule (66).

It seems difficult to account for the rotational barrier but it may probably be connected with the tendency for the unshared p electrons on the two sulphur atoms to overlap when the dihedral angle is 0° or 180° (cf. 35). This overlap must then result in coulombic repulsion between non-bonding electron pairs and hence a potential barrier at the coplanar position with a minimum near 90° (68).

For comparison with related groups it should be mentioned that quantum mechanical calculations have established a dihedral angle of about 100° for the peroxide bond in hydrogen peroxide (69). There exists also an energy barrier, which must be passed to permit rotation from this angle. ROGERS and CAMPBELL have made dipole moment measurements (70) on related aliphatic peroxides, disulphides and diselenides, from which conclusions are drawn about the dihedral angles.

From the above considerations about the stabilized skew configuration of linear disulphides (for a representative figure, see (38)) it can be readily understood that special properties must be attributed to five-membered and to some extent also to six-membered cyclic disulphides. This is well illustrated by the work of AFFLECK and DOUGHERTY (71) on the preparation and relative reactivities of the homologous unsubstituted saturated cyclic disulphides. Larger rings do not require any appreciable deviation from the normal value of the dihedral angle and therefore correspond more closely to the linear disulphides. This is substantiated from the spectrochemical investigations of CALVIN and co-workers (35) and the present author (see below). It is further strengthened by the calorimetric determinations of SUNNER (38).

In this investigation substituted cyclic disulphides have been studied and in particular carboxylic acid derivatives, which are specially suitable owing to their solubility properties. Although substituents stabilize the strained system a certain reactivity is found for the compounds derived from 1,2-dithiolane. A comparison of the properties of the homologous disulphide acids (IV), (I) and (V) and the observations made upon their preparation indicate that the dominating influence within the molecule of the disulphide group decreases distinctly with increasing ring size.

The significant properties of different types of disulphides have been correlated to each other and a common basis obtained from which conclusions can be drawn, thereby extending our knowledge about the cyclic disulphide systems. In order to make the examination more complete the spectrochemical studies include even the isomeric dithio-dibenzoic acids and their dimethyl esters (10).

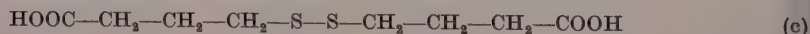
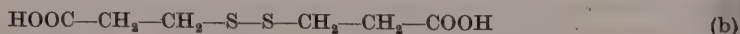
Finally it should be mentioned that photolysis experiments on 1,2-dithiolane have been performed by CALVIN and co-workers (35, 36). Recent work with monochromatic radiation (36) has shown that the number of photons required to break the S-S bond is of the order of unity, which makes reasonable the assumption of a repulsive excited state from which energy may be efficiently transferred. The present author has examined briefly the photolysis of *racem*-1,2-dithiolane-3,5-dicarboxylic acid (11) and of 1,2-dithiolane-4-carboxylic acid. The results imply opening of the rings by S-S bond fission, very likely with a homolytic splitting of the linkage into a biradical, which immediately reacts in some way. No quantitative examinations of the reaction mixtures have been made as yet. According to CALVIN and WHITNEY (36) the biradicals react with the solvent (alcohol).

Ultraviolet absorption

Ultraviolet absorption measurements mainly on aliphatic dialkyl disulphides have previously been reported by e.g. KOCH (72), BAER and CARMACK (73) and by BRANDT,

EMELÉUS and HASZELDINE (74). It is known that non-cyclic disulphides show one characteristic selective absorption in the vacuum ultraviolet region with a maximum at about 200 $m\mu$ and one absorption peak near 250 $m\mu$ in the near ultraviolet region. This characteristic 250 $m\mu$ maximum seems due to the excitation of the non-bonding p -electrons or the πp electrons if we consider the existing overlap of the unshared electron pairs. The present author has recently examined the spectra of some cyclic disulphides (11), and in order to interpret the possible influence of substituents the study was extended to include carboxylic substituted aliphatic disulphides. It was found that dithio-diacetic acid (dithio-diglycolic acid) has only continuous absorption in the wavelength region under question (220–330 $m\mu$) with increasing extinction towards shorter wavelengths. An analogous spectral behaviour is reported by KOCH for di-*tert.* butyl disulphide (72). A carboxylic group thus causes the same effect as the three methyl groups attached to the same carbon atom. As these groups have different modes of inductive electronic effects it was considered likely that the shifts or complete changes were due to steric effects. KOCH had offered such an explanation for the anomalous behaviour of di-*tert.* butyl disulphide. It was, however, stated that an examination of the corresponding diselenides should be valuable for comparison. The selenium atoms are larger than the sulphur atoms (covalent radius : 1.17 Å and 1.04 Å resp. (75)) and substituents attached to the Se–Se group are thus more distant from each other than if they were linked to an S–S group. The spatial interactions of the substituents in a diselenide must therefore be smaller than in a disulphide and it should be possible to study pure electronic effects. An examination of the ultraviolet absorption spectra of some linear diselenides has now been published by BERGSON (76), from which it may be concluded that the diselenides are more suitable for theoretical studies than the disulphides, as their absorption is found at longer wavelengths and the two absorption bands are more separate. The discussion given in reference (11) may now be somewhat modified but also made more precise after comparison with the corresponding diselenide spectra and extension of the disulphide material. From the spectral behaviour of di-*tert.* butyl diselenide (76) BERGSON is able to give an account also for the continuous absorption of the corresponding disulphide. For, if the characteristic absorption band undergoes a hypsochromic shift of the same order of magnitude as that of the diselenide the two absorption bands of di-*tert.* butyl disulphide should be so close together that the resulting absorption would be continuous in the wavelength region examined. The continuous shift of the specific absorption for diselenides and disulphides with different number of C–H bonds (3, 2, 1, 0 resp.) towards shorter wavelengths may be accounted for by assuming hyperconjugation of the C–H σ -orbitals with the orbitals of the selenium or sulphur atom; a recent investigation of the infrared absorption (the C–Se bond) has substantiated this interpretation (77).

The examination of the dicarboxylic substituted disulphides clearly illustrate the different influence of substituents. The substances can be collected in two series as



and





The different types of absorption are given in Table 4.

The spectra of dithio-diacetic acid (a), dithio- α,α' -dipropionic acid (d) and dithio- α,α' -di-*iso*-butyric acid (e) are shown in Fig. 1. The absorption curves for the diastereomers of (d) are nearly identical (11).

From the series (a)–(c) it can be seen that a carboxylic group in the γ -position of the sulphur atoms does not exhibit any influence on the characteristics of the disulphide group. The spectral behaviour of dithio-diacetic acid was discussed in reference (11). This work had indicated that there might be a change in the dihedral angle. The dihedral angle is principally determined by the repulsion of the non-bonding p-electrons and with changed electronic conditions of the disulphide group there should be a small change of the angle. BERGSON has, however, shown that considerable spectral differences occur for diselenides, even when the dihedral angles do not show much variation (76). Although no value of the dihedral angle of any carboxylic substituted disulphide is known, it seems justified to assume analogous behaviour for diselenides and disulphides. The continuous type of ultraviolet absorption for dithio-diacetic acid may therefore be explained by the combined results of a bathochromic shift of the absorption band in the vacuum ultraviolet region (in analogy to diselenium-diacetic acid (76);) and a hypsochromic shift of the usual 250 $\text{m}\mu$ absorption. The effect on the band in the near ultraviolet region is due to the inductive effect of the carboxylic groups, which involves the withdrawal of electrons from the disulphide group. The decrease in mutual repulsion of the electrons causes excitation at higher energy, thus a shift towards shorter wavelengths. An overlap of the two absorption bands gives the continuous spectrum. It can now be pointed out that a comparison of the disulphides (a)–(c) illustrates the influence of the carboxylic group on the disulphide system under analogous hyperconjugative conditions, whereas the series (a), (d), (e) shows the results of hyperconjugation and alkyl inductive effects with unchanged substitution for the carboxylic groups.

Table 4. UV absorption of some disulphide dicarboxylic acids and their salts (220–330 $\text{m}\mu$) (solvent: alcohol and water resp.).

Substance	Type of absorption	
	Acid	Di-Na-salt
(a)	continuous	continuous
(b)	max. 245 $\text{m}\mu$; min. 235 $\text{m}\mu$	max. 246 $\text{m}\mu$; min. 237 $\text{m}\mu$
(c)	max. 249 $\text{m}\mu$; min. 230 $\text{m}\mu$	max. 245 $\text{m}\mu$; min. 232 $\text{m}\mu$
(d)	continuous	continuous
(e)	continuous	continuous

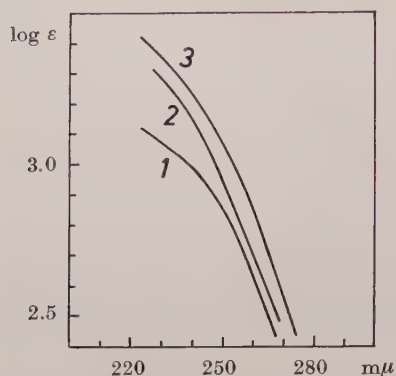


Fig. 1. UV absorption spectra of: (1) dithio-diacetic acid; (2) dithio- α, α' -dipropionic acid (racemic form); (3) dithio- α, α' -di-*iso*-butyric acid. Solvent: alcohol.

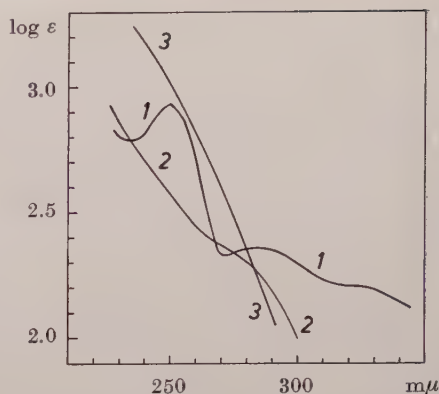


Fig. 2. UV absorption spectra of: (1) *racem*-1,2-dithiolane-3,5-dicarboxylic acid; (2) *racem*-1,2-dithiane-3,6-dicarboxylic acid; (3) *racem*-1,2-dithiepane-3,7-dicarboxylic acid. Solvent: alcohol.

The interpretation given above as to the relationship between the spectral behaviour of disulphides and the substituent influence on the S-S group should also apply for the cyclic derivatives examined in references (11) and (9). The investigation has now been extended to include also *racem*-1,2-dithiepane-3,7-dicarboxylic acid (V) and 1,2-dithiolane-4-carboxylic acid (VI) (and their salts). Fig. 2 shows the UV absorption of the racemic cyclic five-, six- and seven-membered disulphide- α, α' -

Table 5. UV absorption of some cyclic disulphide acids and their salts (220–360 $m\mu$) (solvent: alcohol and water resp.).

Substance	Type of absorption	
	Acid	Na-salt (neutral)
1,2-Dithiolane-4-carboxylic acid	max. 330 $m\mu$; min. 280 $m\mu$	max. 330 $m\mu$; min. 280 $m\mu$
<i>Rac.</i> -1,2-Dithiolane-3,5-dicarboxylic acid	max. 250 $m\mu$; min. 235 $m\mu$ (max. 285 $m\mu$; min. 270 $m\mu$; inflex. 330 $m\mu$)	max. 330 $m\mu$; inflex. 280 $m\mu$; min. 300 $m\mu$ *
<i>Rac.</i> -1,2-Dithiane-3,6-dicarboxylic acid	inflex. c. 280 $m\mu$	inflex. c. 275 $m\mu$
<i>Meso</i> -1,2-Dithiane-3,6-dicarboxylic acid	continuous	inflex. c. 273 $m\mu$
<i>Rac.</i> -1,2-Dithiepane-3,7-dicarboxylic acid	continuous	continuous
<i>Meso</i> -1,2-Dithiepane-3,7-dicarboxylic acid	continuous	continuous

* The acid Na-salt has: inflex. c. 246 $m\mu$; min. 270 $m\mu$; max. 280 $m\mu$; min. 300 $m\mu$; max. c. 320 $m\mu$.

Table 6. UV absorption of some 4-substituted 1,2-dithiolanes (220–380 $m\mu$) (solvent: alcohol).

Substance	Type of absorption
1,2-Dithiolane-4-carboxylic acid	max. 330 $m\mu$; min. 280 $m\mu$
4,4-Dimethyl-1,2-dithiolane	max. 331 $m\mu$; min. 284 $m\mu$
4,4-Bis-hydroxymethyl-1,2-dithiolane	max. 328 $m\mu$; min. 285 $m\mu$; inflex. 255 $m\mu$
1,2-Dithiolane-4-semicarbazone	max. 330 $m\mu$; min. 302 $m\mu$; max. 236 $m\mu$
2,3-Dithia-(4,5)-spirodecane	max. 329 $m\mu$; min. 283 $m\mu$; inflex. c. 260 $m\mu$
2,3,7,8-Tetrathia-(4,4)-spirononane	max. 333 $m\mu$; min. 287 $m\mu$
2,6,7-Trithia-(3,4)-spirooctane	max. 354 $m\mu$; min. 303 $m\mu$; max. 264 $m\mu$; min. 257 $m\mu$

dicarboxylic acids. The spectral properties of *meso*-(V) are given in reference (11). The different types of absorption of the cyclic disulphides investigated are collected in Tables 5 and 6.

The ultraviolet absorption spectra of 1,2-dithiolane-4-carboxylic acid (VI) and 2,3,7,8-tetrathia-(4,4)-spirononane (VIII) are illustrated in Fig. 3. 1,2-Dithiolane-3-carboxylic acid has an absorption peak at 280 $m\mu$ (62).

It should be observed that the absorption curves of the five- and six-membered saturated cyclic disulphides have been recorded by CALVIN and co-workers (35) together with that of 4-thioctic acid (4,8-dithio-*n*-octanoic acid), representing the seven-membered ring system. A displacement of the absorption top to longer wavelengths is observed, when the size of the disulphide ring diminishes. This can be interpreted as due to the changed electronic conditions around the S-S group. Thus the *cis* position of the disulphide group in 1,2-dithiolane derivatives involves a compression of the *p*-electron orbitals, which results in an increased repulsion between the two non-bonding *p*-electron pairs and hence excitation by less energy. It appears from references (9) and (11) and from Tables 5 and 6 that the influence of the substituents resembles that of the linear disulphides. Special attention is drawn to the interesting fact that the spectral behaviour of 1,2-dithiolane itself and the 4-substituted derivatives are closely related.

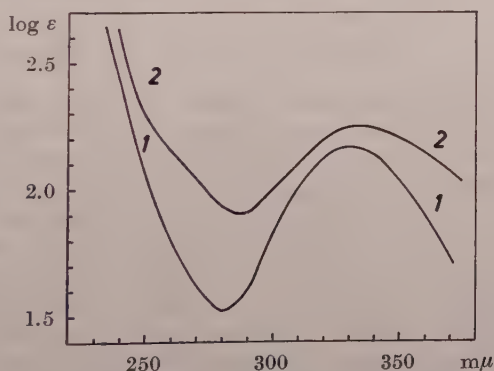


Fig. 3. UV absorption spectra of: (1) 1,2-dithiolane-4-carboxylic acid; (2) 2,3,7,8-tetrathia-(4,4)-spirononane. Solvent: alcohol.

The ultraviolet absorption curve of *racem*-1,2-dithiolane-3,5-dicarboxylic acid (IV) is interesting since in addition to the 250 $m\mu$ peak it also contains two absorption bands of lower intensity at longer wavelengths. It seems difficult to give an explanation for this observation.

It is most interesting to note the appreciable change in UV absorption of *racem*-(IV) upon neutralization of the acid (11). As no analogous effects have been found by the present author for 1,2-dithiolane-4-carboxylic acid and 6-thioctic acid (the spectra for the acid and salt are similar), it seems justified to ascribe the observed change to the substitution of carboxylic groups at the α -position to the disulphide group. However, a detailed interpretation cannot be given at present.

The spectrochemical investigation has been extended to include also the study of isomeric dithio-dibenzoic acids and their dimethyl esters (10). The ways of approaching the problem are certainly somewhat different for the aromatic derivatives on one side and the non-aromatic on the other, but by this examination the effect of conjugation of the disulphide group is clearly demonstrated. The *m*-compounds show less intense absorption than the *o*- and *p*-isomers. Displacements towards longer wavelengths are observed for the latter isomers. A recent paper by KISS and MUTH (78) describes the UV absorption spectrum of *o,o'*-dithio-dibenzoic acid in addition to those of the related sulphide, selenide and diselenide. Our experimental results seem consistent.

Infrared absorption

The applications of infrared research to organic sulphur compounds have hitherto been rather few and so this investigation has in view not only a comparison of the homologous linear and cyclic disulphide dicarboxylic acids and different 1,2-dithiolane derivatives but also the provision of further material for qualitative deductions as to the properties of the S-S group. It is certainly true that the sulphur-sulphur bond is best studied using Raman spectroscopy but by careful analysis even the infrared absorption spectra recorded in the potassium bromide prism region may give valuable information (7, 9-11, 44). The disulphide stretching frequency is generally assigned the region 400-500 cm^{-1} (25.0-20.0 μ) (79) and the S-S peaks are in general regarded as ill-defined and difficult to characterize due to low intensities (small changes in the dipole moment upon stretching of the group). For the aromatic disulphides CYMERMAN and WILLIS (80) have reported the region 430-490 cm^{-1} (23.2-20.4 μ). The carbon-sulphur stretching frequency is assigned to 600-700 cm^{-1} (16.7-14.3 μ).

Interpretations of the absorption curves have not been possible for all the disulphides investigated but conclusions may often be drawn from a comparison of related substances, especially if one of the compounds shows a simple spectrum. A typical example is found for the diastereomers of dithio- α,α' -dipropionic acid (44), where the extreme simplicity of the spectrum of the racemic isomer allows assignments of the bands to be made for both the acids. In general, care should be exercised in interpretation and reservations entered in case of uncertainties. A considerable extension of the materials examined in reference (11) has now been made. The frequencies ascribed to the carbon-sulphur and sulphur-sulphur stretching vibrations for linear and cyclic disulphide dicarboxylic acids are collected in Tables 7 and 8 respectively. The wavelengths of the absorption bands for dithio-diacetic acid, dithio- β,β' -dipropionic acid and dithio- α,α' -di-*iso*-butyric acid within the potassium

Table 7. Frequencies ascribed to the C-S and S-S stretching vibrations for some linear disulphide dicarboxylic acids.

Substance	Wavelength, μ		Wavenumber, cm^{-1}	
	C-S	S-S	C-S	S-S
Dithio-diacetic acid	15.05	c. 21.8*	664	c. 459*
Rac.-Dithio- α,α' -dipropionic acid	15.10	20.5	662	488
Meso-Dithio- α,α' -dipropionic acid	14.82; 15.10	20.6	675; 662	485
Dithio- β,β' -dipropionic acid	15.25 and (or) 15.60	19.0	656; 641	526
Dithio- α,α' -di-iso-butyric acid	15.50	19.65	645	509
Dithio- γ,γ' -di-n-butyric acid	†	†	†	†

* Uncertainty involved. † No assignment possible.

Table 8. Frequencies ascribed to the C-S and S-S stretching vibrations for some cyclic disulphide dicarboxylic acids.

Substance	Wavelength, μ		Wavenumber, cm^{-1}	
	C S	S-S	C-S	S-S
Rac.-1,2-dithiolane-3,5-dicarboxylic acid	15.06	18.57	664	539
Rac.-1,2-dithiane-3,6-dicarboxylic acid	15.10	20.70	662	483
Meso-1,2-dithiane-3,6-dicarboxylic acid	14.50 and 14.72	19.45*	690; 679	514*
Rac.-1,2-dithiepane-3,7-dicarboxylic acid	†	†	†	†
Meso-1,2-dithiepane-3,7-dicarboxylic acid	†	†	†	†

* Uncertainty involved. † No assignment possible.

 Table 9. The infrared absorption of some linear dithio-dicarboxylic acids. (11–23 μ ; KBr phase).

b = broad; vs = very strong; s = strong; m = medium; w = weak; sh = shoulder.

Dithio-diacetic acid			Dithio- β,β' -dipropionic acid			Dithio- α,α' -di-iso-butyric acid		
Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band	Wave-length in μ	Wave-number in cm^{-1}	Character of the band
2.85	778	w	12.30	813	vs	12.40	806	vs
3.04	767	w	13.18	759	w	13.10	763	w
5.05	664	s	15.25	656	s	15.50	645	s
7.43	574	m	15.60	641	s	18.12	552	vs
7.71	565	m	18.15	551	m	18.5	541	sh
9.7	508	w b	19.0	526	m b	19.65	509	m
1.8	c. 459	m b						

bromide prism region are given in Table 9. The frequencies ascribed to the stretching vibrations of the S-S group in some 1,2-dithiolane derivatives are reported in Table 2, reference (9).

Unfortunately no assignments were possible for the 1,2-dithiepane derivatives due to the complicated nature of the absorption curves. This seems due to the structural vibrations which are more pronounced with increasing number of methylene groups in the ring or the chain. In addition to the monomeric *racem*- and *meso*-1,2-dithiepane-3,7-dicarboxylic acid the polymer described in reference (4) has been investigated. The outlines of the curves are rather similar but distinct differences occur in the region $7-9\mu$ ("finger print region"), where the polymer has a set of sharp bands. In the potassium bromide prism region, where the carbon-sulphur and sulphur-sulphur bands usually occur, a certain similarity is found. The behaviour of the S-S group in larger rings should in reality resemble roughly that of well-defined polymers.

It seems possible to conclude from the above that the frequencies of the carbon-sulphur stretching vibrations for the disulphide acids vary within a rather close interval which is consistent with that postulated in general. It should be noted that the shift towards lower frequencies for dithio- α,α' -di-*iso*-butyric acid as compared with dithio-diacetic acid is in agreement with the results of SHEPPARD (81) that the C-S stretching frequency for *tert.* alkyl sulphides is appreciably lower than that of similar compounds with primary alkyl groups. These differences may be interpreted in terms of changes in the force constants of the linkages. Further support is thereby given to the above-mentioned hyperconjugation (cf. the discussion of UV spectra) of the C-H σ -orbitals with the orbitals of the sulphur atom. For the 4-substituted 1,2-dithiolanes and the aromatic disulphides no assignments as to the C-S linkages have been possible due to structural vibrations at frequencies in the vicinity of those expected for carbon-sulphur bonds.

This investigation has shown that the region, which is generally assigned to the disulphide stretching frequency ($400-500\text{ cm}^{-1}$), should be somewhat modified. In fact, the disulphide acids have their S-S absorption bands at frequencies near or higher than 500 cm^{-1} . As could be expected special properties have been found for the 1,2-dithiolane derivatives. The disulphide bands occur at higher frequencies

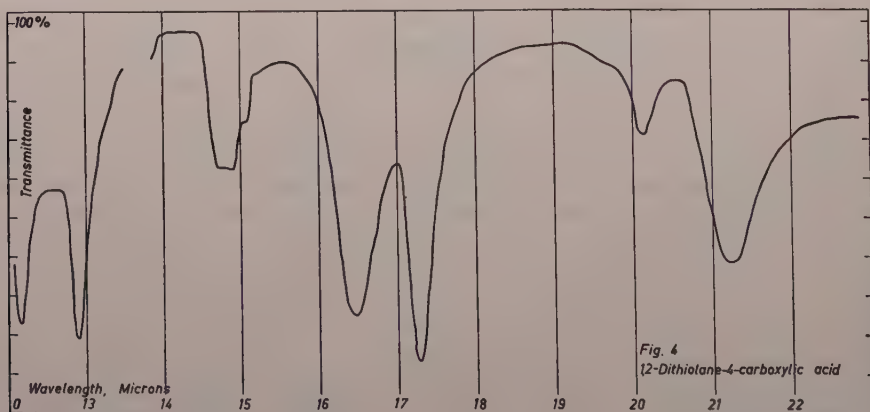


Fig. 4.

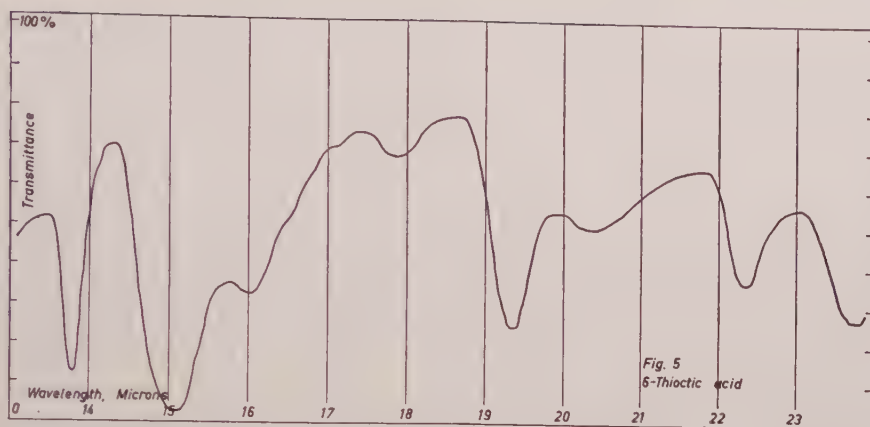


Fig. 5.

than those of other disulphides and this reflects well the electronic conditions around the S-S group in the five-membered ring. It should be noted that the 4-substituted 1,2-dithiolanes are in general characterized by higher S-S frequencies than the 3-substituted. However, variation occurs to some extent even for closely related compounds. As this investigation seems to be the first involving infrared studies on cyclic disulphides (the IR spectrum of 6-thioctic acid in the NaCl region has been reported (22)) with regard to the carbon-sulphur and sulphur-sulphur bonds, it may be justified to present the IR spectra of 1,2-dithiolane-4-carboxylic acid and 6-thioctic acid (Figs. 4 and 5) in the potassium bromide region, although no unambiguous interpretation of the bands can be offered at present due to the rather complicated nature of the curves. This seems especially well-grounded, if we consider the possible biochemical importance of these substances.

For the aromatic disulphides the S-S stretching vibration frequencies have been found within or close to the region proposed ($430\text{--}490\text{ cm}^{-1}$). The reader is directed to Figs. 3 and 4 in reference (10) for detailed information.

Polarimetry

The enantiomorphs of 1,2-dithiolane-3,5-dicarboxylic acid show a very high molecular rotation in different solvents (abs. ethanol, $[\text{M}]_{\text{D}}^{25} = +1092^\circ$ and -1088° resp.) (5). It should be noted that the rotation in aqueous medium depends on the acidity of the solution, which can be directly connected with the observed gradual change of the character of the ultraviolet absorption spectrum upon neutralization of the acid (11). The preliminary resolution experiments of *racem*-1,2-dithiepane-3,7-dicarboxylic acid also accounts for the high rotatory power. As maximum optical activity has not yet been obtained, further discussion must be postponed. It is, however, interesting to note that the specific rotation is higher than that of the corresponding 1,2-dithiane derivative (14). This observation may indicate an alternating effect within the homologous series or may be connected with the more symmetrical structure of the six-membered disulphide acid. The high rotatory power of cyclic disulphides has been somewhat discussed in reference (5). Attention is called to the racemization tendency of the enantiomorphs of 1,2-dithiolane-3,5-dicarboxylic acid (5).

Very interesting polarimetric investigations of the redox process mercaptan-disulphide (82) and of the diselenide dismutations (83) have been made by FREDGA. The marked difference in activity of disulphide and mercaptan makes the use of polarimetry advantageous for the establishment of redox processes. The reaction between *racem*-1,2-dithiolane-3,5-dicarboxylic acid (IV) and the related dimercaptan has been preliminarily studied. The kinetic interpretation of the results must, however, wait until further experiments are made.

It may finally be pointed out that no reaction between the cyclic five-membered disulphide acid (IV) and dithio-diacetic acid could be obtained under conditions, in which the corresponding reaction occurs for diselenides. The difference in reactivity in this respect between the Se-Se and S-S groups (83) thus seems to include even the strained 1,2-dithiolane system.

Polarography

In an attempt to find a method of comparing the ring stability of the cyclic disulphide acids having different ring sizes a polarographic investigation has recently been undertaken in collaboration with Dr. B. NYGÅRD (84). A brief note that the

Table 10. Half-wave potentials vs. S.C.E. at pH = 2.2 (citrate buffer) for some cyclic disulphide acids (conc. = 1.00×10^{-3} M).

Substance	Half-wave potential, volt
<i>Rac</i> -1,2-Dithiolane-3,5-dicarboxylic acid	-0.33*
<i>Rac</i> -1,2-Dithiane-3,6-dicarboxylic acid	-0.76
<i>Meso</i> -1,2-Dithiane-3,6-dicarboxylic acid	-0.75
<i>Rac</i> -1,2-Dithiepane-3,7-dicarboxylic acid	-0.92
<i>Meso</i> -1,2-Dithiepane-3,7-dicarboxylic acid	-0.93
6-Thioctic acid	-0.36
1,2-Dithiolane-3-carboxylic acid	-0.37
1,2-Dithiolane-4-carboxylic acid	-0.37

* A bad wave in acetate buffer, pH = 4.6: — 0.44 V.

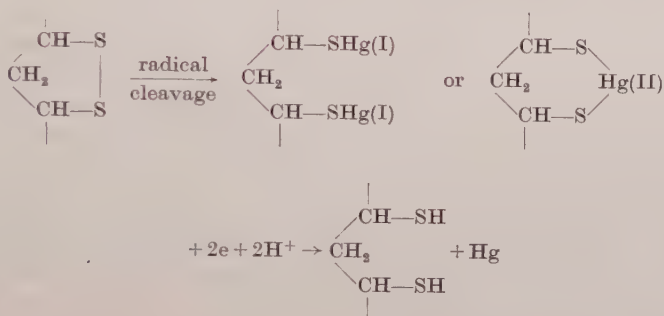
Table 11. Half-wave potentials vs. S.C.E. in different standard buffer solutions for some 4-substituted 1,2-dithiolanes (conc. 1.00×10^{-3} M).

Substance	Half-wave potential, volt			
	Citrate buffer pH = 2.2	Acetate buffer pH = 4.6	Phosphate buffer pH = 7.0	Borate buffer pH = 9.2
1,2-Dithiolane-4-carboxylic acid . .	-0.37	—	-0.65*	-0.76*
4,4-Bis-hydroxymethyl-1,2-dithiolane	-0.31	-0.44	-0.68	-0.70
4,4-Dimethyl-1,2-dithiolane	-0.34	—	-0.68	-0.78
2,3-Dithia-(4,5)-spirodecane	-0.34	-0.49	-0.64	-0.79
2,6,7-Trithia-(3,4)-spirooctane . .	-0.33	-0.48	-0.62	-0.77
2,3,7,8-Tetrathia-(4,4)-spirononane .	-0.40	-0.55	-0.68	-0.83

* The wave is somewhat deformed.

disulphide linkage in 6-thioctic acid is reducible at the dropping mercury electrode (21) seems to be the only report in the literature about the polarographic behaviour of cyclic disulphides. A number of substances have been examined and the half-wave potentials vs. the saturated calomel electrode (S.C.E.) are collected in Tables 10 and 11.

At pH = 2.2 all the 1,2-dithiolane derivatives give very good waves which are reversible in character. The wave heights and the relations $\log [i/(i_d - i)]$ vs. the applied potentials E indicate a two-electron reduction. Analogous to the polarographic behaviour of some aliphatic disulphides (cystine, glutathione) the waves obtained seem to be due to the reduction of Hg complexes which are formed on the drop at the rupture of the S-S linkage. The electroreduction should thus proceed according to, e.g.



The six- and seven-membered cyclic disulphide dicarboxylic acids give polarographic waves only in acid solution and the waves are rather drawn out indicating irreversible electroreduction. In both the cases the irreversibility is more pronounced for the meso acids than for the racemic isomers.

The fact that the polarographic effect seems based upon the intermediate Hg complex formation makes a direct comparison inadequate for the establishment of a possible difference in ring stability. On the other hand, a study of the outlines of the curves may indicate roughly the degree of irreversibility which is connected with the primary reaction, i.e. with the homolytic cleavage of the S-S bond. It can thus be concluded that *racem*-1,2-dithiolane-3,5-dicarboxylic acid reacts more readily than the homologous acids with larger rings. In fact, the waves of the 1,2-dithiepane derivatives resemble those obtained for dithio-di-acetic acid (irreversible in character) (85). The polarographic data are thus in agreement with the above results.

It is obvious from the foregoing that the polarographic effects of the 1,2-dithiolane derivatives are of special interest. It should be noted that 4-substituted 1,2-dithiolanes give in general good waves over the whole pH range. Only the carboxylic substituted derivative shows slight deformations at higher pH. On the other hand, it can be seen from Fig. 6 that in neutral medium the wave of *racem*-1,2-dithiolane-3,5-dicarboxylic acid is quite deformed and at higher pH no waves are found. This observation should probably be connected with the above discussed effect of anions on the characteristic UV absorption spectrum and as 6-thioctic acid has been found to give polarographic waves similar to those of the 4-substituted 1,2-dithiolanes, it seems justified to postulate a considerable effect of a carboxylic group only if it is substituted at the carbon atom in α -position to the S-S group. This establishment

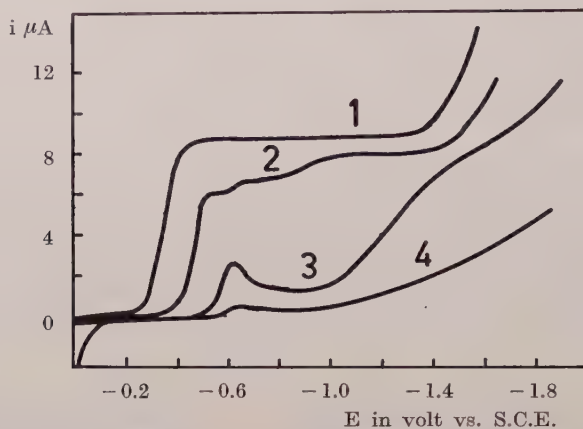


Fig. 6. Polarograms of 1.00×10^{-3} M *racem*-1,2-dithiolane-3,5-dicarboxylic acid in various buffers: (1) citrate buffer, pH=2.2; (2) acetate buffer, pH=4.6; (3) phosphate buffer, pH=7.0; (4) borate buffer, pH=9.2. (The solutions contain 0.005 % gelatin as max.-suppressor and 0.1 M KNO_3 .)

is supported by the behaviour of 1,2-dithiolane-3-carboxylic acid. The salt of the five-membered cyclic disulphide dicarboxylic acid is in some way stabilized against the attack by mercury. It is difficult to understand the real significance of this observation, but there seems to exist a correlation between this behaviour and the spectral properties.

It should finally be noted that diselenides are characterized by reversible effects over the whole pH range. Diseleno-diacetic acid has been studied in detail by NYGÅRD (86). The formation of electroreducible Hg complexes on the mercury drop is more easily realized for selenium compounds. Such a difference in reactivity with mercury has already been established by FREDGA for the aliphatic S-S and Se-Se groups (87, 88). In this respect the 1,2-dithiolane derivatives resemble the diselenides and show greater affinity to mercury than disulphides in general except those involving heterocyclic structures (89).

Steric relations

If the optically active 1,2-dithiolane-3,5-dicarboxylic acid (IV) can be sterically related to the glyceric aldehyde system, a method is offered for the establishment of the absolute configuration of 6-thioctic acid. It therefore seemed important to establish the configuration of (IV).

In the problem of determining the configurative relationships between optically active substances the formation of quasi-racemic compounds plays an important role. A molecular compound formed from two optically active substances is considered as a quasi-racemic compound if each component forms a molecular compound only with one antipode of the other. It is then obvious that the steric factors are of primary importance in the formation of the compounds. The quasi-racemate method has been thoroughly investigated and successfully applied to a number of steric problems by FREDGA and collaborators (90, 91). Although X-ray diagrams often prove to be of value (92), the existence of the quasi-racemic compounds is

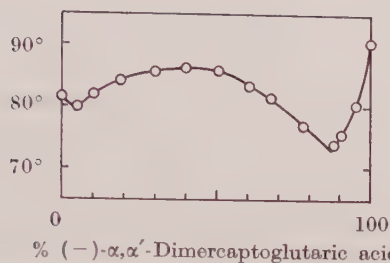


Fig. 7. Melting point diagram for the system (+)- α, α' -dimethylglutaric acid and (-)- α, α' -dimercaptoglutaric acid.

usually demonstrated by means of melting point curves. A further method, involving the use of infrared absorption measurements to determine the existence of quasi-racemates has recently been presented by ROSENBERG and SCHOTTE (43).

It is difficult to find a substance with established absolute configuration for direct comparison to the enantiomorphs of (IV). However, reduction of the disulphide acids give optically active α, α' -dimercaptoglutaric acids (IX) (the product has opposite mode of rotation) in reactions which do not affect the centres of asymmetry (5). These mercapto acids show a pronounced tendency for racemate formation, and as preliminary work on the melting point diagram of the enantiomorphs indicated similarity to the curve given by FREDGA (93) for the antipodes of α, α' -dimethylglutaric acid (X), it seemed justified to try to correlate α, α' -dimercaptoglutaric acid and the dimethyl compound by the quasi-racemate method. Optically active α, α' -dimethylglutaric acid (X) has been connected to the glyceric aldehyde system by FREDGA (94). However, a complete thermal analysis of the phase diagrams should be avoided, since it has been found that the melting point of the mercapto acid depends upon the rate of heating due to partial decomposition. The method of instantaneous melting (95) was applied but nevertheless reproducible results could only be obtained for the system (+)-(X) and (-)-(IX), where the

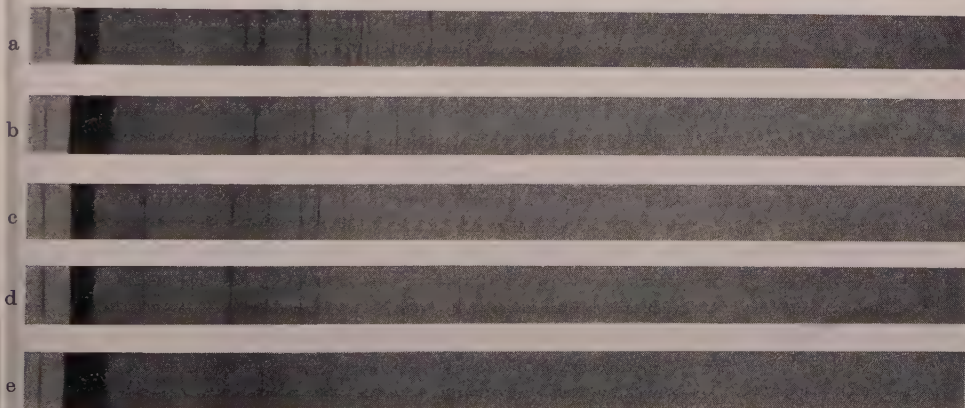
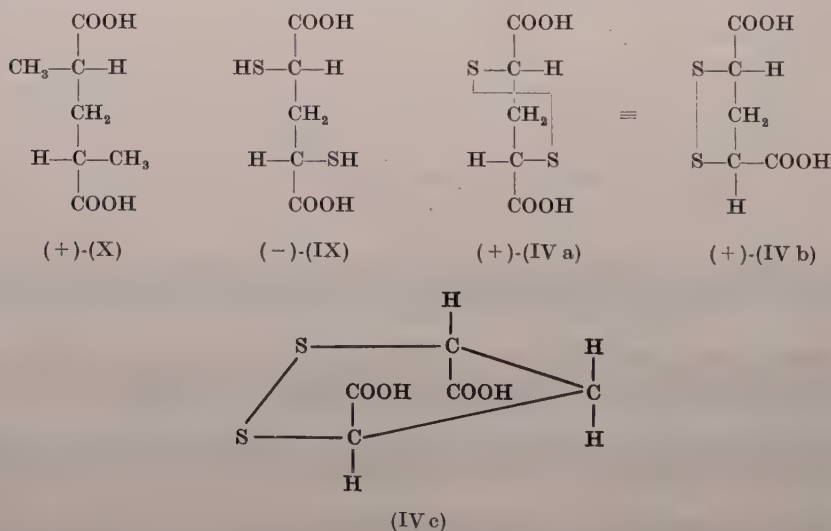


Fig. 8. Guinier powder photographs (Cu-K α radiation) of: (a) *racem*- α, α' -dimercaptoglutaric acid; (b) (+)- α, α' -dimercaptoglutaric acid; (c) (+)- α, α' -dimethylglutaric acid; (d) equimolar amounts of (+)- α, α' -dimethylglutaric acid and (-)- α, α' -dimercaptoglutaric acid; (e) equimolar amounts of (+)- α, α' -dimethylglutaric acid and (+)- α, α' -dimercaptoglutaric acid.

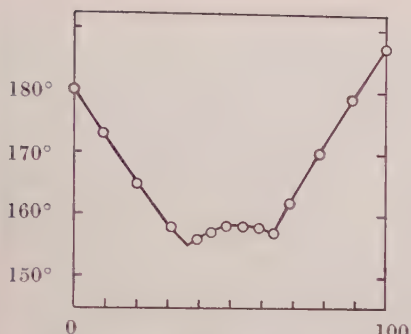
melting points were in general lower than those of the systems (+)- and (-)-(IX), and (+)-(X) and (+)-(IX). The approximate outlines of the two latter diagrams resembled each other and suggested molecular compounds in the ratio 1:1 melting 40° – 50° higher than the optically active components. However, as the curves were quantitatively unreliable they are omitted here. Fig. 7 shows the remaining diagram, (+)-(X) and (-)-(IX), which seems to consist of a solid solution between 0 % and about 85 % of (IX) with a minimum at about 5 % and a maximum at about 50 %. The rest of the curve is of the eutectic type. This interpretation has been confirmed by studies of contact preparations (96) and by means of X-ray powder photographs (see Fig. 8, *c* and *d*). It may be mentioned that a melting point curve of the same type is described by PETERSSON (97) for the system (+)- α -phenylhydrocinnamic acid and (+)- α -(2-thienyl)-hydrocinnamic acid. The above results indicate the connection between direction of rotation and configuration but it should be stressed that in this case definite conclusions must not be drawn from melting point curves only. The systems have therefore been investigated with X-rays. The different powder photographs are shown in Fig. 8. The true racemic character of *racem*-(IX) is obvious from comparisons of *a* and *b*. If we compare *c* with *d* and *e*, there is a close similarity between *c* and *d*, whereas differences in line distributions occur for *d* and *e*. This is consistent with the existence of a quasi-racemic compound between equivalent amounts of (+)-(X) and (+)-(IX). Infrared absorption studies support this view.

On the basis of the results obtained it seems possible to relate (X) to the sulphur compounds (IX) and (IV) by the following stereo-formulae:



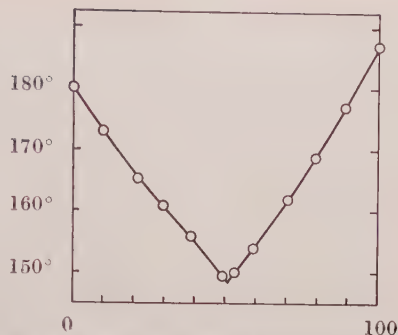
The formulae (IV a) and (IV b) are perspective reproduced in (IV c).

There are several cases known where quasi-racemic compounds can be formed from components which are structurally identical except for the replacement of a CH_2 group in the one by an S atom in the other (98). However, the replacement of the S-S group in linear disulphides by CH_2 - CH_2 involves a considerable structural change due to the skew configuration of disulphides and the changed bond lengths. The differences are obviously large enough to prevent the formation of a quasi-



% (-)-1,2-Dithiolane-3,5-dicarboxylic acid

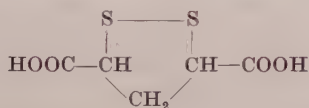
Fig. 9. Melting point diagram for the system (+)-thiophane-2,5-dicarboxylic acid and (-)-1,2-dithiolane-3,5-dicarboxylic acid.



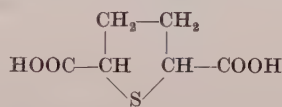
% (+)-1,2-Dithiolane-3,5-dicarboxylic acid

Fig. 10. Melting point diagram for the system (+)-thiophane-2,5-dicarboxylic acid and (+)-1,2-dithiolane-3,5-dicarboxylic acid.

racemate. This has been established by HEDLUND in the case of the system dithio- α,α' -dipropionic acid and α,α' -dimethyladipic acid (99). The present investigation has shown that of the factors controlling the above-mentioned structural change, the configuration of the S-S group is by far the most important. This can be seen from Figs. 9 and 10, where the melting point curves of the systems (-)-1,2-dithiolane-3,5-dicarboxylic acid (IV), (+)-thiophane-2,5-dicarboxylic acid (XI) and (+)-(IV), (+)-(XI) respectively are given. The diagram for the enantiomorphs of (IV) is reported in reference (5) and that of the antipodes of (XI) in reference (12).



(IV)



(XI)

(XI) may be considered as formally derived from (IV) by the replacement of an S-S group by CH_2-CH_2 and a CH_2 by an S atom. As the acids with the same mode of rotation give a curve of eutectic type (Fig. 10) and those of opposite rotation form a molecular compound in the ratio 1:1, we may conclude that the acids with the same direction of rotation have the "same configuration". Furthermore the probable existence of a quasi-racemic compound between (-)-(IV) and (+)-(XI) is indicated by infrared absorption measurements. The spectrum of the mixture of equivalent amounts of (-)-(IV) and (+)-(XI) within the sodium prism region resembles closely that of *racem*-(IV) (11). Fig. 11 represents part of the infrared spectra of both the equimolar mixtures (+), (-) and (+), (+), recorded between 6.5μ and 9.0μ , where pronounced differences occur.

This result illustrates well the consequence of the *cis*-configuration (dihedral angle probably near 0°) of the disulphide group in 1,2-dithiolane derivatives with their nearly planar ring structure.

In some cases information about the steric relations between similarly constituted compounds may be obtained by measuring the optical activity in a number of

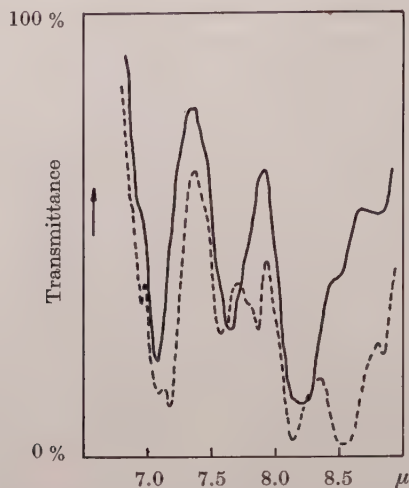


Fig. 11. IR absorption spectra (6.5–9.0 μ) of equimolar amounts of: (+)-thiophane-2,5-dicarboxylic acid and (–)-1,2-dithiolane-3,5-dicarboxylic acid; (+)-thiophane-2,5-dicarboxylic acid and (+)-1,2-dithiolane-3,5-dicarboxylic acid (dotted curve).

Table 12. The molecular rotation, $[M]_D^{25}$, in different solvents for (–)- α, α' -dimercaptoglutaric acid and (–)- α, α' -dimercaptoadipic acid.

Solvent	Molecular rotation	
	(–)- α, α' -Dimercapto-glutaric acid (Ref. (5))	(–)- α, α' -Dimercapto-adipic acid (Ref. (14))
Water (both carboxylic groups neutralized)	– 60°	– 39°
Glacial acetic acid	– 115°	– 147.5°
Ethanol (abs.)	– 143°	– 126°
Ethyl acetate	– 162°	– 147.5°

Table 13. The molecular rotation, $[M]_D^{25}$, in different solvents for (+)-1,2-dithiolane-3,5-dicarboxylic and (–)-1,2-dithiane-3,6-dicarboxylic acid.

Solvent	Molecular rotation	
	(+)-1,2-Dithiolane-3,5-dicarboxylic acid	(–)-1,2-Dithiane-3,6-dicarboxylic acid
Water (neutral salt)	+ 688°	– 699°
Ethanol (abs.)	+ 1092°	– 405.5°
Ethyl acetate	+ 1095°	– 354.5°
Hydrochloric acid (dil.)	+ 1407°*	+ 263°†
Glacial acetic acid	+ 1470°	– 95°

* 2N HCl. † 0.4N HCl.

different solvents (100). Compounds with the same absolute configuration should than show the same mode of variation of the optical activity. The adipic and glutaric acid derivatives are compared in Tables 12 and 13 with respect to the mercaptans and disulphides. A certain similarity is found for the disulphides but due to the difference in dihedral angle of the S-S groups in the five- and six-membered cyclic compounds it is possible that the structural differences are too large to allow any conclusions from the activity data.

Structure of some polysulphides

As the formation of monosulphides in the above mentioned syntheses of cyclic disulphides from dibromo acids and alkali disulphides might be connected with the existence of an unstable intermediate with coordinately linked sulphur, it seemed justified to somewhat consider the structure of some polysulphides. This investigation (7) was performed on tri- and tetrasulphides of spirane structures (54-56) which is especially well-grounded as the related disulphides are 1,2-dithiolane derivatives (9). The results, which indicate unbranched sulphur chains, are given in reference (7). In addition to the further references given there, it may be mentioned that analogous conclusions have recently been drawn by means of tracer technique (101), with X-ray analyses (102) and Raman spectroscopy (103) as regards the structure of some other organic di- and polysulphides.

Additional support to the structures postulated for the polysulphides examined in reference (7) has now been obtained by polarographic studies. The compounds give well-defined polarographic waves at half-wave potentials (vs. S.C.E.), which are close to those given for the 1,2-dithiolane derivatives in Table 11. The heights of the waves are about 50 % greater than for the disulphides, and the polarographic behaviour suggests a structure with three similar sulphur atoms, which is consistent

with the postulated $\begin{array}{c} \diagup \text{S} \diagdown \\ \diagdown \text{S} \diagup \end{array}$ but not with $\begin{array}{c} \diagdown \text{S} - \text{S} \diagup \\ \downarrow \\ \text{S} \end{array}$. A structure of the latter

type must give rise to more than one polarographic wave. The half-wave potentials are given in Table 14.

Table 14. Half-wave potentials vs. S.C.E. of two polysulphides in different buffers (conc. $1.00 \times 10^{-3} M$).

Substance	Half-wave potential, volt		
	Citrate buffer pH = 2.2	Phosphate buffer pH = 7.0	Borate buffer pH = 9.2
2,6,7,8-Tetrathia-(3,5)-spirononane	-0.39	-0.67	-0.77
5,5-Dimethyl-1,2,3-trithiane	-0.40	-0.68	-0.78

Experimental

For further details about the techniques of the *ultraviolet* and *infrared absorption measurements* and the *polarographic work* the reader is referred to references (7), (9–11) and (84).

The preparations for the *melting point diagrams* were made by dissolving exactly weighed amounts of the components in acetone of high purity. The solvent was evaporated at room temperature and the residue removed *in vacuo* after powdering. The melting points were determined in capillary tubes with preheated apparatus or rapid heating (calibrated thermometer; electrically heated apparatus except for the determinations with instantaneous melting, when a sulphuric acid bath (of the usual type) was used).

X-ray powder photographs were taken in a focusing camera of the GUINIER (104) type with Cu-K_α radiation. The time of exposure was three hours. The preparations were obtained as above.

Dithio- α,α' -di-iso-butyric acid.—The substance was recrystallized from water (105). M.p. 198° .

Anal. Subst., 65.48 mg; 7.88 ml of 0.0696N NaOH. Equ. wt. Calc. 119.2; Found 119.4.

1,2-Dithiolane-4-carboxylic acid.—The method of preparation is outlined above. The details will be given in reference (58). The acid was recrystallized from benzene-cyclohexane. M.p. 76.5° – 77.5° .

Anal. Subst., 33.20 mg; CO_2 , 38.89 mg; H_2O , 11.61 mg.

„ „ 18.25 mg; 6.86 ml of 0.0709N NaOH.

„ „ 78.41 mg; 7.32 ml of 0.0709N NaOH.

$\text{C}_4\text{H}_6\text{O}_2\text{S}_2$ (150.22) Calc. C 31.98; H 4.03; S 42.68; Equ. wt. 150.2; Mol. wt. 150.

Found C 31.96; H 3.92; S 42.72; Equ. wt. 151.1; Mol. wt. 146.

The molecular weight has been determined by the ebullioscopic method with acetone as a solvent (cf. 9).

The related dimercaptan was obtained by reduction of the disulphide with zinc in ammoniacal solution. M.p. 57° – 60° . (Cf. also (106)).

Anal. Subst., 36.97 mg; 4.89 ml of 0.0999 N J_2 . Equ. wt. Calc. 76.1; Found 75.7.

Summary

Racem-1,2-dithiolane-3,5-dicarboxylic acid, the enanthiomorphs of 1,2-dithiepane-3,7-dicarboxylic acid and some related compounds have been prepared.

The synthesis, separation into diastereomers and configuration of the starting materials, α,α' -dibromoglutaric and -pimelic acid, have been discussed.

A survey of methods for the preparation of α,α' -dimercapto-dicarboxylic acids is given. The possible modes of reaction in the preparation of cyclic disulphides from halogenides and alkali disulphide are pointed out.

1,2-Dithiolane-4-carboxylic acid, possibly the disulphide present in asparagus, has been prepared.

Special attention has been paid to the study of the properties of cyclic disulphides with different ring sizes by physico-chemical methods.

The influence of substituents like the carboxylic group on the characteristic UV spectrum of disulphides has been interpreted as due to inductive effects. 3- and 4-Substituted 1,2-dithiolanes have been examined; a close similarity between 1,2-dithiolane itself and the 4-substituted derivatives is established.

Infrared absorption studies of the C-S, S-S, and C=S linkages are presented. Higher frequencies than those in general accepted for disulphides have been ascribed to the S-S stretching vibrations in the 1,2-dithiolane derivatives. The infrared absorption behaviour of the carbonyl groups in acid salts has been briefly treated.

Polarographic studies have been made on the five-, six- and seven-membered cyclic disulphide acids. The 1,2-dithiolane derivatives (including 6-thioctic acid) give good polarographic waves which resemble those of linear diselenides. The influence of the substituents on the mercury attack of the S-S linkage at different pH is indicated for the 1,2-dithiolanes.

The steric relationship between 1,2-dithiolane-3,5-dicarboxylic acid and α,α' -dimercaptoglutaric acid to α,α' -dimethylglutaric acid has been established by means of X-ray powder photographs and thereby a connection is given to the glyceric aldehyde system (formulae p. 462).

A quasi-racemate has been obtained between (+)-thiophane-2,5-dicarboxylic acid and (-)-1,2-dithiolane-3,5-dicarboxylic acid. The possibility of replacing $\text{CH}_2\text{-CH}_2$ by S-S is directly connected with the *cis* conformation of the S-S group and the nearly planar structure of the 1,2-dithiolane ring.

The structure of some cyclic polysulphides has been discussed. The improbability of branched sulphur chains is pointed out referring to results obtained by means of UV and IR absorption and polarography.

The special character of the 1,2-dithiolane ring as determined by the above methods in comparison with disulphides in general should finally be stressed.

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Self-diffusion in zinc stannate and stannic oxide

By ROLAND LINDNER and OLLE ENQVIST

With 2 figures in the text

The powder reaction between stannic oxide and zinc oxide, leading to the formation of the "inversed" 4-2-spinel, SnZn_2O_4 , has been investigated by Aleixandre and Verduch (1). The two chief conclusions of that work are the following:

1. By using the initial part of the curve: reaction yield vs. time, a constant for the phase boundary reaction is obtained with a temperature dependence according to an activation energy of 74 700 cal/mole. As this value happens to equal that found for the self-diffusion of zinc in zinc oxide (2), the conclusion is drawn that the self-diffusion in zinc oxide should be the rate-determining step.

2. Further, the observation has been reported that the reaction layer formed between pellets of the oxides of zinc and tin is located on one side of the original interface, i.e. only on the side of the stannic oxide. This supports the assumption of predominant diffusion of zinc and oxygen through the reaction layer. In connection with a larger program dealing with the mechanism of spinel formation (3) in the solid state, it seemed worthwhile to look more in detail into this matter.

The first assumption mentioned above is somewhat dubious, as it seems improbable that in this case the state within the interior of one of the reactive components should have any large effect on the reaction, which is expected to proceed chiefly by diffusion through the reaction product. Besides, the details of boundary reactions between solid phases are up to now insufficient: usually the partial process called "phase boundary reaction" consists to a great deal of an accelerated diffusion along grain boundaries and dislocations. Only if a linear time function can be established over a longer time (but usually not for yields higher than a few percent), one can assume that processes as the actual phase boundary reaction or the velocity of formation of a new phase (nucleation and grain growth) can be rate determining.

We used the value given by Aleixandre and Verduch in their Fig. 3 to recalculate the reaction rate according to the procedure of Serin and Ellickson (4). This gives fairly straight lines (for $\pi^2 Dt/R^2$ as function of t) for the temperatures 995°, 1040°, and 1055°C, whilst at 1015° a weak concave reflection and at 1085° and 1140° a weak convex reflection results. We conclude that within experimental error a rate-determining diffusion process may be existent. The diffusion constants thus calculated can be arranged around a straight line in the usual graph: $\log D = f(1/T)$, at least as far as lower temperatures are concerned. The activation energy for diffusion according to this procedure is 75 000 cal and the preexponential factor lies with a value of about 2 within reasonable limits. (Cf. Fig. 3.)

Thus the assumption that diffusion through the reaction layer is rate determining should be examined in detail.

Experimental

Pellets of stannic oxide and zinc stannate were obtained by sintering the compacted powder at temperatures of 1400–1450°C, a comparatively short heat treatment (about 15 minutes) being essential in the case of the spinel pellets which otherwise lost zinc oxide by evaporation and decreased in density. The spinel had been pre-formed by the reaction of the finely divided powder mixture at temperatures of about 1100°C.

In spite of the rigorous heat treatment the density of the sintered pellets was not very satisfactory (about 4.0 in the case of stannic oxide and at about 4.2 in the case of the spinel), which involves an appreciable amount of porosity. Nevertheless, the values obtained should to a certain extent represent volume diffusion, as could be shown in similar cases of spinel formation and self-diffusion.

As radioactive tracers we used zinc-65 (half life 250 days) and a mixture of different tin isotopes with the masses 119, 121, 123, 125, all produced by neutron irradiation of tin in the Norwegian reactor. The purity of the radiotracers has been examined by measuring the absorption of the radiation in aluminium.

The self-diffusion in zinc stannate and stannic oxide

The following methods were used: The method of the exchange of radioactive and inactive atoms between the respective sintered pellets kept into close contact with each other at high temperatures (5). In the case of the self-diffusion of zinc some experiments were performed using the method of the decrease in β -activity measured at the surface of the sample, the initial condition being a thin layer of radioactive zinc oxide condensed on that sample after evaporation of the metal in vacuum (2). The results obtained for the self-diffusion of zinc and tin are reproduced in Fig. 1 in the usual way: $\log D = f(1/T)$. The self-diffusion of zinc is considerably higher in the temperature range investigated, the respective equation being $D = 37 \exp(-76300/RT) \text{ cm}^2 \text{ sec}^{-1}$, whilst the self-diffusion of tin in the spinel can be reproduced by the expression $D = 2.3 \times 10^5 \exp(-109000/RT) \text{ cm}^2 \text{ sec}^{-1}$.

We measured also the self-diffusion of tin in stannic oxide in the temperature range of about 1000–1260°C. A rather high activation energy was found (cf. Fig. 2), the expression being $D = 10^6 \exp(-118700 \pm 3700/RT) \text{ cm}^2 \text{ sec}^{-1}$ (as calculated with the method of the least squares).

The reaction constants of the spinel formation

As the determination of the direction of migration within the reaction layer according to the method of the marked initial boundary (6) by Alexandre and Verduch based on only one experiment at a comparatively low temperature (1000°C) and short reaction time (70 hrs), which probably resulted in a layer only 5–10 microns thick, we thought it advisable to repeat this experiment at higher temperatures, using a 2 micron thick platinum foil marker. An experiment at 1150°C during 3 weeks yielded a reaction layer of about 75 microns thickness. After the experiment the following observations were made: The platinum foil was attacked by the solids, the platinum partly dissolved and spread as a blackish compound throughout the reaction layer.

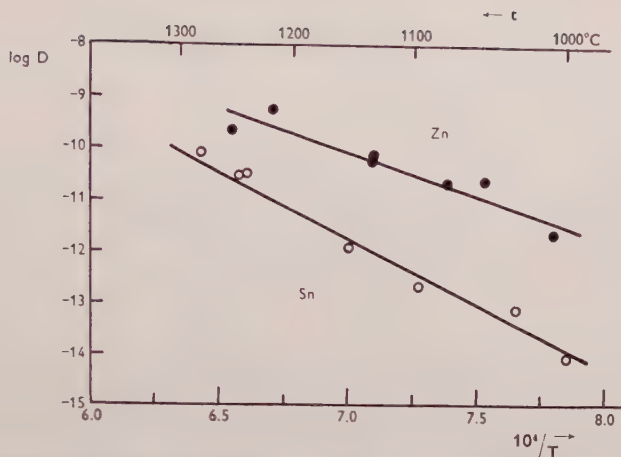


Fig. 1. Self-diffusion constants for zinc and stannic ions in zinc stannate as function of temperature.

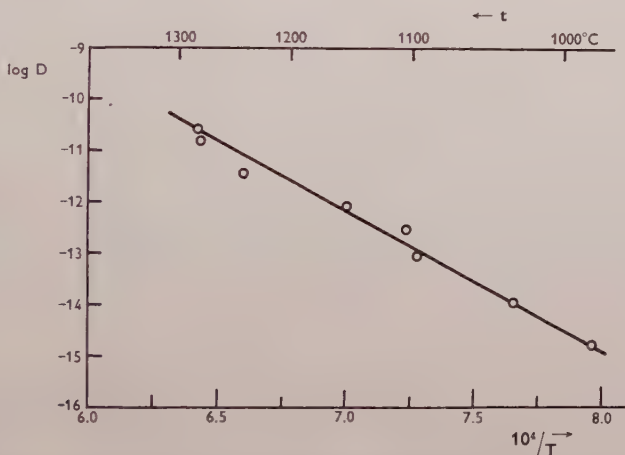


Fig. 2. Self-diffusion of stannic ions in stannic oxide as function of temperature.

Thus it is not yet (future experiments will use Jr-192-markers) possible to decide if the marker of the original interface is located on top of the reaction layer (as assumed by Alexandre and Verduch), or if it is embedded in the middle of the reaction layer as it should be according to the Wagnerreaction mechanism (7), assuming diffusion of zinc ions and stannic ions through the reaction layer in mutual direction.

Another possibility to obtain information about the rate determining process seemed to us an improved determination of the reaction constant using sintered pellets as reactants and thus working with known contact areas. The values for the reaction constants at different temperatures (circles) are (calculated in the dimension $\text{cm}^2 \text{sec}^{-1}$, i.e. the same dimension as the self-diffusion constants) reproduced in Fig. 3 together with the self-diffusion constants of zinc and tin (dotted lines). As can be observed the reaction constants are located nearer to the self-diffusion constant of tin

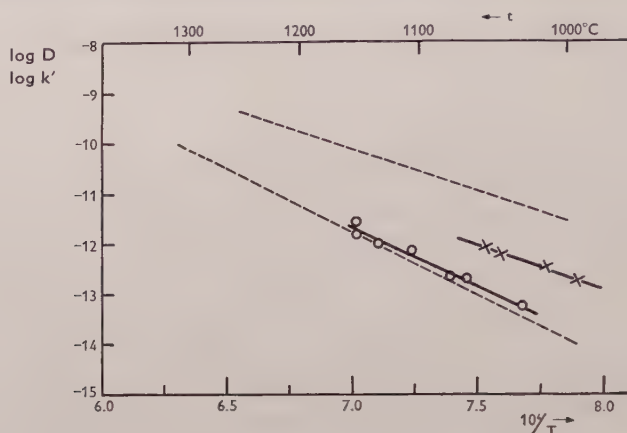


Fig. 3. Comparison of reaction and self-diffusion constants in the system $\text{ZnO} + \text{SnO}_2$.

(lower dotted line) than to that of zinc. This result is understandable when considering the expression for the reaction constant given by Wagner (7) which contains as essential expression for the reaction constant the term $[(D_1 \cdot D_2)/(D_1 + D_2)]$, all other terms (concentrations, valencies, equivalent volume, difference of the chemical potentials at the phase boundaries of the reaction layer) practically compensating each other in this special case. As it can easily be shown the value of the self-diffusion constant of the less mobile ion species determines the reaction rate (if $D_1 \gg D_2$). As shown in Fig. 3 this seems to be the case in the system investigated, the reaction constant being similar to the diffusion constant of stannic ions. The figure contains, besides, the value for the reaction constant calculated according to Serin and Ellickson from data given in the work by Alexandre and Verduch (1) (crosses). These values are higher than ours, which, however, might be explained by the higher degree in irreversible lattice defects influencing powder reactions.

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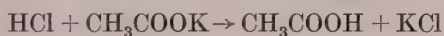
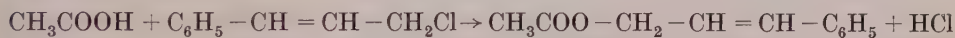
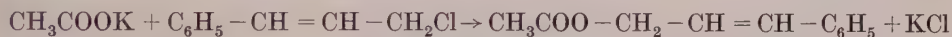
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The effect of changing medium upon the rate of alkylation

By SIGVARD WIDEQVIST

Organic compounds having mobile hydrogen atoms usually react with alkyl halides in the presence of a base. Ordinarily the organic compound at first forms a salt by reaction with the base, and the salt reacts then with the alkyl halide, yielding alkali halide and alkylated product. Numerous examples can be given for the reaction between alkyl halides and alkali salts of tautomeric substances like β -diketones, β -ketoesters, and malonic esters. However, little is known about the reaction between alkyl halides and the free β -keto-compounds. According to Acree [1] this reaction is very slow, because the tautomeric substances are such weak acids that only few anions are formed which can react with the alkyl halide. According to Brändström [2] the chelate enol compounds of the free β -ketoesters and β -diketones cannot be alkylated, because the hydrogen atom in the chelate ring cannot hold more than two groups. It could, however, be questioned, if the free ester or diketone would be more easily alkylated in the presence of an acceptor for the hydrogen halide which is produced during the reaction. It can thus be mentioned that cinnamyl chloride, when heated with a solution of potassium acetate in glacial acetic acid, reacts with both the salt and the free acetic acid, the reaction product being cinnamyl acetate. The potassium acetate also acts as an acceptor for the hydrogen chloride produced in the latter reaction:



If the cinnamyl chloride is denoted RCl and the acetic acid HAc, we find the following differential equation for the reaction velocity

$$d[\text{Cl}^-]/dt = k_1[\text{RCl}][\text{HAc}] + k_2[\text{RCl}][\text{KAc}]. \quad (1)$$

As the concentration of the free acetic acid is constant, the reaction would follow a mixed second order plus pseudo first order kinetics. This is also verified by Meissenheimer and Beutler [3].

In the present investigation the reaction between sodiacetoacetic ester and methyl iodide in ethyl alcohol was studied. In order to find out if the free ester could also be alkylated, the reaction was performed in the presence of excess acetoacetic ester. The sodium enolate would thus act both as reactant and as acceptor for the hydrogen iodide formed. It was found that the rate of reaction increased with the concentration

of free ester. The reaction was, however, purely second order, and the velocity of the reaction could be expressed by the differential equation

$$-d[\text{alkali}]/dt = (k_1 + k_2[\text{ester}])[\text{alkali}][\text{methyl iodide}]. \quad (2)$$

This indicates that the free ester is not alkylated to any appreciable extent. The same results were obtained when the sodium salt of methyl acetoacetate was alkylated with methyl iodide in methanol.

It is now generally accepted that a reaction goes through an "activated complex", and that the rate of reaction is proportional to the concentration of this complex. It can therefore be called into question, if the free ester is also incorporated with the activated complex.

The influence of excess ester on the rate of reaction between sodiomalonic ester and ethyl bromide in alcohol has previously been studied by Pearson [4]. The reaction was followed conductometrically, and Pearson found that the rate of alkylation increased with the concentration of free malonic ester. He assumed that this effect was due to the equilibrium between ethoxide ions and ester anions:



If HE denotes the free ester, E⁻ the ester anion, ROH the alcohol, and RO⁻ the alkoxide ion, we get the equilibrium condition

$$[\text{E}^-][\text{ROH}]/[\text{RO}^-][\text{HE}] = K \quad (3)$$

or

$$[\text{E}^-] = K ([\text{HE}]/[\text{ROH}]) [\text{RO}^-]. \quad (4)$$

If [HE] and [ROH] can be regarded as constant during the reaction we can put

$$[\text{E}^-] = n [\text{RO}^-]. \quad (5)$$

Further we get

$$[\text{E}^-] + [\text{RO}^-] = a - z \quad (6)$$

where $a - z$ is the total concentration of alkoxide and enolate.

When an alkyl halide, R'X, reacts with the equilibrium mixture of alkoxide and enolate, the reaction products are alkylated ester, R'E, and dialkyl ether, R'OR. If the concentration of alkyl halide is denoted $b - z$ and the concentrations of R'E and R'OR are denoted x and y , respectively, we get for the rate of reaction the differential equations

$$dx/dt = k_1[\text{E}^-](b - z) \quad (7)$$

$$dy/dt = k_2[\text{RO}^-](b - z). \quad (8)$$

From eq. (5) and (6) we obtain

$$[\text{E}^-] = \frac{n}{n+1} (a - z) \quad \text{and} \quad [\text{RO}^-] = \frac{1}{n+1} (a - z)$$

and thus

$$dx/dt = k_1 \frac{n}{n+1} (a-z)(b-z) \quad (7a)$$

$$dy/dt = k_2 \frac{1}{n+1} (a-z)(b-z). \quad (8a)$$

But $z = x + y$. On addition of eq. (7a) and (8a) we thus get

$$dz/dt = [(k_1 n + k_2)/(n+1)](a-z)(b-z). \quad (9)$$

As seen from eq. (9) a pure second order reaction should also be obtained in the case of an equilibrium between two different alkali compounds, if the reaction is followed by measurements of the residual alkali, $a-z$, or the alkali halide, z , formed during the reaction. The distribution of the reaction products is

$$x/y = n k_1/k_2. \quad (10)$$

Eq. (9) is, strictly speaking, valid only when the alkali enolate and the alkoxide are completely dissociated into ions or when the degree of ionization can be regarded as constant during the reaction.

The validity of eq. (9) was tested against the reaction between methyl iodide and the equilibrium mixture of sodium ethoxide and sodium methoxide in equal volumes of ethanol and methanol. A perfect second order reaction was obtained (Table 18).

In the investigation of the alkylation of sodiomalonic ester Pearson determined the amount of ester converted into ester anions in the following way: Resistances of solutions containing a fixed concentration of sodium ethoxide and varying amounts of malonic ester were measured at 25°. The resistance increased with the concentration of malonic ester, according to Pearson, because of the replacement of the ethoxide ions by the less mobile malonic ester anions. Because of the weak acidity of the ester this change should not be complete for any solution experimentally available. However, if R_0 is the resistance, when the malonic ester concentration is zero and R the resistance, when the ester concentration is c , then a plot of $R/(R-R_0)$ against $1/c$ gives a straight line which can be extrapolated to $1/c$ equal to zero. This gives the value of R_∞ which is the resistance of a hypothetical solution of infinite ester concentration and in which all the sodium ethoxide has been converted into sodiomalonic ester. From values of R_∞ , R_0 , and R the fraction of ethoxide ions converted to ester anions was found and the equilibrium constant K in eq. (3) determined. The following results were reported for the measurements on a solution containing 0.0279*M* sodium ethoxide:

[Malonic ester] mol./l.	Resistance ohms
0.0000	3 606
0.0099	3 976
0.0199	4 386
0.0793	6 260
0.1585	8 192
∞	13 910 (calcd.)

It seems to have been assumed that the sodiomalonic ester is present exclusively in the form of ions and that the limiting resistance R_∞ corresponds to a 0.0279*M* sodiomalonic ester solution with ethyl alcohol as solvent. If eq. (4) is considered, we find, however, that the ethoxide ion concentration becomes equal to zero also, when

the alcohol concentration, $[\text{ROH}]$, is zero. This is exactly what happens when malonic ester is gradually substituted for the alcohol. It can therefore be discussed whether the increase of the resistance is not also a consequence of changing the medium. The resistance R_∞ should thus be referred to a solution with malonic ester as solvent instead of alcohol. This point of view is based upon the following. According to the measurements of Barak and Hartley [5] the limiting equivalent conductances in ethanol at 25° for the sodium ion and the ethoxide ion are 18.7 and 23.3, respectively, and the limiting conductance of sodium ethoxide is thus 42.0. The limiting equivalent conductance of sodiomalonic ester, however, cannot be less than that of the sodium ion alone, namely 18.7. A rough estimation of the equivalent conductances at the concentration 0.0279*M* can be made by use of Shedlovsky's [6] extension of Onsager's equation which for alcohol at 25° takes the form

$$\Lambda = \Lambda_0 - (\Lambda/\Lambda_0) (1.35 \Lambda_0 + 89.4) c^{\frac{1}{2}}. \quad (11)$$

The constants in eq. (11) are taken from Davies [7]. With the assumption of complete dissociation of the electrolytes we obtain for sodium ethoxide the equivalent conductance 26.6 and as a lower limit for sodiomalonic ester 9.24. The resistance of a 0.0279*M* sodium ethoxide solution was 3606 ohms. The resistance of a 0.0279*M* sodiomalonic ester solution can thus not exceed $(26.6/9.24) \times 3606 = 10,370$ ohms, if ethyl alcohol is the solvent, but Pearson found 13,910 ohms. This discrepancy might partly depend on alterations in the ionization of the electrolytes, because the addition of malonic ester depresses the dielectric constant of the solution. The dielectric constant of alcohol is 24 at 25° (Davies [7]) and that of the malonic ester 8.4 at 20.8° (Dobrosserdow [8]).

In alcoholic solution sodiomalonic ester is probably a weak electrolyte which is only partly ionized:



Unfortunately there are no measurements available on the electrolytic conductivity of sodiomalonic ester in ethanolic solution at various concentrations. Such measurements have, however, been performed on other ester enolates. As an example the conductivity data of sodioacetoacetic ester in alcohol at 25°, reported by White [9], can be mentioned. From these data the present author has calculated the ionization constant of the sodioacetoacetic ester according to Ostwald's dilution law (Table 1).

Table 1. The ionization constant of sodioacetoacetic ester in ethanol at 25°. (From the data of White [9].)

v	Λ	α	$K \times 10^3$
16	6.691	0.1818	2.53
32	8.96	.2434	2.45
64	12.288	.3338	2.61
128	15.81	.4295	2.53
256	19.71	.5355	2.41
512	24.34	.6612	2.52
1 024	28.74	.7808	2.72
∞	36.81		

Mean value $K = 2.54 \times 10^{-3}$.

The existence of an ionization equilibrium seems thus to be beyond question. Pearson, however, neglects the ion association and assumes that only the ester anions are alkylated, but according to Acree's [10] theory of alkylation both the ions and the undissociated molecules are alkylated. This point of view has recently been suggested also by Brändström [2], although he assumes that the undissociated molecules are present in the form of metal chelate compounds.

In the present investigation on the methylation of acetoacetic ester and methyl acetoacetate similar results were obtained as those obtained by Pearson in the alkylation of malonic ester, when the influence of excess ester upon the rate of alkylation was studied. It seemed, however, important to investigate also the influence of the solvent. This was performed on the reaction between methyl iodide and methyl acetoacetate in a mixture of methanol and other solvents. The addition of dioxane thus caused an increase of the rate of reaction. This is also what could be expected, as the dielectric constant of dioxane, 2.1, is less than that of methanol, 31.5 (Harned and Owen [11]); according to the theory of reaction rates a depression of the dielectric constant of the medium shall increase the rate of reaction, if the reaction can be assumed to take place between an ion and a neutral molecule (Glasstone, Laidler, and Eyring [12]). The same results were obtained when the reaction between sodium methoxide and methyl iodide was studied in mixtures of methanol and dioxane. The methylation of methyl acetoacetate was also studied in a medium having somewhat higher dielectric constant than methanol, namely a mixture of methanol and acetonitrile. The dielectric constant of acetonitrile is 35.8 at 20° (Walden [13]). However, the addition of acetonitrile also caused an increase of the reaction rate. This indicates that the influence of the dielectric constant is less important. The specific rate of the reaction increased linearly with volume content of the added solvent and the effect was almost exactly the same for dioxane and acetonitrile. It could therefore be thought that the increase of the rate was due to an increase of the enolate content because of the depression of the methanol concentration. (Compare eq. (4).) In order to ascertain if this interpretation was correct, the reaction was studied in a medium containing a large amount of free methyl acetoacetate. The effect was, however, exactly the same as in the case of acetonitrile and dioxane.

Experimental

Methyl iodide, ethyl acetoacetate, and methyl acetoacetate were redistilled commercial products.

Absolute ethyl alcohol was prepared by dissolving sodium in 99.5 % alcohol, boiling with diethyl phthalate and finally distilling through a column.

The methanol was Merck's analytical reagent which was used without further purification.

Dioxane, Eastman, was purified by boiling with sodium for several hours and distillation.

Acetonitrile, Merck, was boiled with phosphorus pentoxide and distilled through a column.

The alkoxides were prepared by dissolving clean sodium in the appropriate alcohol.

The reaction mixtures were kept at $25.0 \pm 0.02^\circ\text{C}$ by means of a water thermostat. In order to avoid undesirable effects of the medium all the reaction mixtures contained the same amount of methyl iodide, namely 1 ml per 50 ml of solution. Samples were taken with a 5 ml pipette and poured into a known quantity of hydrochloric acid.

The excess acid was titrated with standardized 0.1*N* carbonate-free sodium hydroxide, using methyl red as an indicator. The time for the first sample was chosen as arbitrary zero time.

The following symbols are used:

- a_0 = concentration of sodium enolate at the time of mixing,
 a = concentration at zero time,
 $a-x$ = concentration at time t ,
 b_0 = concentration of methyl iodide at the time of mixing,
 b = concentration at zero time,
 $b-x$ = concentration at time t ,
 c = concentration of free ester,
 t = time in minutes from the zero time,
 k = reaction velocity constant,
 $k^* = \frac{1}{t} \log \frac{a(b-x)}{b(a-x)} = 0.4343 (b-a) k$.

All concentrations are expressed in moles/litre.

The measurements can be divided into the following groups:

I. The reaction between the sodium enolate of acetoacetic ester and methyl iodide in ethanol at fixed concentrations of enolate and methyl iodide and different concentrations of free ester (Tables 2-6).

II. The reaction between the sodium enolate of methyl acetoacetate and methyl iodide in methanol under the same conditions as above (Tables 7-10).

III. The reaction between the sodium enolate of methyl acetoacetate and methyl iodide in methanol at fixed concentrations of enolate, methyl iodide, and free ester. The composition of the medium was changed by addition of dioxane, acetonitrile, and methyl acetoacetate. In the last case the "solvent ester" is distinguished from "the concentration of free ester". (Tables 11-15.).

IV. The reaction of methyl iodide with sodium ethoxide and sodium methoxide in ethanol and methanol, respectively, and with sodium ethoxide plus sodium methoxide in ethanol plus methanol. The reaction of sodium methoxide was studied at various concentrations of dioxane. (Tables 17-23.)

Table 2. The methylation of ethyl acetoacetate in ethanol.

$$c = 0.05155$$

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0990	0.3236	
10	.0757	.3003	8.44
20	.0589	.2835	8.41
30	.0467	.2713	8.32
40	.0369	.2615	8.39
50	.0300	.2546	8.30
60	.0238	.2484	8.42

$$k = 8.58 \times 10^{-2}$$

Table 3. The methylation of ethyl acetoacetate in ethanol.

$$c = 0.2112$$

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0961	0.3208	
10	.0740	.2987	8.27
20	.0564	.2812	8.70
30	.0433	.2680	8.95
40	.0347	.2594	8.76
50	.0271	.2518	8.89
60	.0227	.2475	8.56

$$k = 8.92 \times 10^{-2}$$

Table 4. The methylation of ethyl acetoacetate in ethanol.

$$c = 0.3599$$

t	$a-x$	$b-x$	$k^* \cdot 10^3$
0	0.0890	0.3136	
10	.0667	.2913	9.32
20	.0505	.2751	9.45
30	.0391	.2637	9.38
40	.0307	.2553	9.31
50	.0244	.2490	9.25
60	.0188	.2434	9.43

$$k = 9.59 \times 10^{-2}$$

Table 5. The methylation of ethyl acetoacetate in ethanol.

$$c = 0.5289$$

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0930	0.3176	
10	.0683	.2929	9.92
20	.0519	.2765	9.66
30	.0399	.2645	9.60
40	.0307	.2553	9.65
50	.0240	.2486	9.66
60	.0184	.2430	9.80

$$k = 9.94 \times 10^{-2}$$

Table 6. The methylation of ethyl acetoacetate in ethanol. The relation between the rate of reaction and the concentration of free ester.

a_0	b_0	c	$k \times 10^2$	
			found	calcd.
0.1022	.03268	0.0516	8.58	8.55
0.1021	0.3268	0.2112	8.92	9.03
0.1022	0.3268	0.3599	9.59	9.47
0.1022	0.3268	0.5289	9.94	9.98

The results from Tables 2-5 are summarized in Table 6. The reaction velocity constant can be expressed as a linear function of the concentration of the free ester. Thus we obtain for acetoacetic ester in ethanol

$$k = 0.08395 + 0.02997 c. \quad (12)$$

The values of k , calculated from eq. (12), are recorded in column 5 of Table 6 and show good agreement with the experimental values. For $c = 0$ we get $k = 8.40 \times 10^{-2}$.

Table 7. The methylation of methyl acetoacetate in methanol.

$$c = 0.0770$$

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.1025	0.3237	
10	.0904	.3117	3.79
20	.0809	.3021	3.65
30	.0719	.2931	3.70
40	.0639	.2851	3.75
50	.0569	.2781	3.79
60	.0513	.2725	3.76
90	.0379	.2591	3.72

$$k = 3.89 \times 10^{-2}$$

Table 8. The methylation of methyl acetoacetate in methanol.

$$c = 0.2623$$

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0987	0.3194	
10	.0866	.3074	3.98
20	.0769	.2976	3.89
30	.0679	.2887	3.95
40	.0605	.2813	3.94
50	.0537	.2745	3.97
60	.0471	.2687	3.98

$$k = 4.14 \times 10^{-2}$$

Table 9. The methylation of methyl acetoacetate in methanol.

$$c = 0.6273$$

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0999	0.3211	
10	.0864	.3077	4.44
20	.0749	.2961	4.50
30	.0657	.2869	4.44
40	.0581	.2793	4.37
50	.0509	.2721	4.42
60	.0447	.2659	4.45
90	.0313	.2526	4.44

$$k = 4.61 \times 10^{-2}$$

Table 10. The methylation of methyl acetoacetate in methanol. The relation between the rate of reaction and the concentration of free ester.

a_0	b_0	c	$k \times 10^2$	
			found	calcd.
0.1056	0.3268	0.0770	3.89	3.90
0.1054	0.3262	0.2623	4.14	4.14
0.1056	0.3268	0.6273	4.61	4.61

It can be noted that Brändström [2] found the velocity constant 7.25×10^{-2} for the same reaction at 25° , when *tert*-butanol was used as solvent.

The results from Tables 7-9 are summarized in Table 10. For the reaction between the sodium salt of methyl acetoacetate and methyl iodide in methanol the rate constant is related to the concentration of free ester by the formula

$$k = 0.03797 + 0.01305 c. \quad (13)$$

The values of k calculated from eq. (13) are recorded in column 5 of Table 10. For $c = 0$ we obtain $k = 3.80 \times 10^{-2}$ which is in good agreement with the value 3.70×10^{-2} , reported by Brändström [2]. The slight difference can be attributed to an effect of the medium caused by the methyl iodide. In the present investigation the concentration of methyl iodide was more than 50 per cent higher than that used by Brändström.

The results from Tables 11-15, showing the effect of changing the medium on the rate of the reaction between methyl iodide and the sodium salt of methyl acetoacetate, are summarized in Table 16. The medium was methanol which was gradually diluted with acetonitrile, dioxane, and methyl acetoacetate. It is found that the velocity constant is largely independent of the nature of the added solvent but increases linearly with its volume content. The following relation is obtained

$$k = 0.0389 + 0.001515 p, \quad (14)$$

where p is the volume per cent of the added solvent. The parameters in eq. (14) were calculated from the values of k for pure methanol and 40% dioxane. The calculated

Table 11. The methylation of methyl acetoacetate in 10 % acetonitrile.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0974	0.3175	
10	.0827	.3027	5.07
20	.0709	.2909	5.01
30	.0609	.2810	5.04
40	.0521	.2722	5.14
50	.0459	.2660	4.81
60	.0397	.2598	5.04
90	.0268	.2468	5.02

$$k = 5.26 \times 10^{-2}$$

Table 12. The methylation of methyl acetoacetate in 20 % acetonitrile.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0988	0.3205	
10	.0797	.3013	6.68
20	.0651	.2867	6.65
30	.0533	.2750	6.72
40	.0443	.2660	6.68
50	.0371	.2588	6.65
60	.0311	.2528	6.64
90	.0186	.2402	6.68

$$k = 6.92 \times 10^{-2}$$

Table 13. The methylation of methyl acetoacetate in 20 % methyl acetoacetate.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0986	0.3186	
10	.0795	.2995	6.67
20	.0655	.2855	6.50
30	.0541	.2742	6.51
40	.0453	.2654	6.45
50	.0375	.2576	6.54
60	.0315	.2516	6.54
90	.0192	.2392	6.52

$$k = 6.82 \times 10^{-2}$$

Table 14. The methylation of methyl acetoacetate in 20 % dioxane.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0920	0.3137	
10	.0747	.2963	6.61
20	.0611	.2827	6.64
30	.0501	.2718	6.73
40	.0419	.2636	6.65
50	.0345	.2562	6.75
60	.0294	.2510	6.66

$$k = 6.96 \times 10^{-2}$$

Table 15. The methylation of methyl acetoacetate in 40 % dioxane.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0991	0.3208	
5	.0845	.3061	9.85
10	.0733	.2949	9.48
15	.0633	.2849	9.56
20	.0553	.2770	9.49
25	.0481	.2698	9.55
30	.0421	.2638	9.56
40	.0321	.2538	9.69
60	.0200	.2416	9.55

$$k = 9.95 \times 10^{-2}$$

Table 16. The methylation of methyl acetoacetate. The influence of medium upon the rate of reaction.

a_0	b_0	c	Medium	$k \times 10^2$	
				found	calcd.
0.1056	0.3268	0.0770	Methanol	3.89	(3.89)
0.1066	0.3267	0.0766	Methanol/10 % Acetonitrile	5.26	5.40
0.1050	0.3267	0.0754	Methanol/20 % Acetonitrile	6.92	6.92
0.1066	0.3267	0.0778	Methanol/20 % Methyl acetoacetate	6.82	6.92
0.1050	0.3267	0.0738	Methanol/20 % Dioxane	6.96	6.92
0.1050	0.3267	0.0736	Methanol/40 % Dioxane	9.95	(9.95)

Table 17. The reaction between methyl iodide and sodium ethoxide in ethanol.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0955	0.3252	
10	.0729	.2975	8.58
20	.0565	.2811	8.58
30	.0447	.2693	8.49
40	.0349	.2595	8.64
50	.0284	.2530	8.51
60	.0226	.2472	8.58

$$k = 8.78 \times 10^{-2}$$

Table 19. The reaction between methyl iodide and sodium methoxide in methanol.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.1043	0.3256	
10	.0988	.3200	1.61
30	.0890	.3103	1.60
50	.0805	.3017	1.58
80	.0697	.2921	1.60

$$k = 1.67 \times 10^{-2}$$

Table 18. The reaction between methyl iodide and the equilibrium mixture of sodium methoxide and sodium ethoxide in equal volumes of methanol and ethanol.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.1039	0.3239	
10	.0941	.3141	2.96
20	.0855	.3055	2.96
30	.0779	.2979	2.96
40	.0715	.2915	2.92
50	.0649	.2849	2.98
60	.0596	.2797	2.95
90	.0462	.2663	2.96

$$k = 3.10 \times 10^{-2}$$

Table 20. The reaction between methyl iodide and sodium methoxide in 20 % dioxane.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.1018	0.3235	
15	.0874	.3091	3.09
30	.0753	.2969	3.13
45	.0657	.2873	3.09
60	.0571	.2788	3.11
75	.0497	.2714	3.14
90	.0437	.2654	3.12

$$k = 3.25 \times 10^{-2}$$

Table 21. The reaction between methyl iodide and sodium methoxide in 40 % dioxane.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0990	0.3207	
10	.0831	.3047	5.42
20	.0701	.2917	5.45
30	.0595	.2811	5.46
40	.0511	.2728	5.43
50	.0435	.2652	5.49
60	.0377	.2594	5.45

$$k = 5.67 \times 10^{-2}$$

Table 22. The reaction between methyl iodide and sodium methoxide in 60 % dioxane.

t	$a-x$	$b-x$	$k^* \times 10^3$
0	0.0988	0.3205	
10	.0767	.2983	7.91
20	.0597	.2813	8.11
30	.0475	.2692	8.08
40	.0377	.2594	8.16
50	.0307	.2524	8.07
60	.0252	.2468	8.02

$$k = 8.36 \times 10^{-2}$$

Table 23. The reaction between methyl iodide and sodium methoxide. The influence of the medium upon the rate of reaction.

a_0	b_0	Medium	$k \times 10^3$
0.1056	0.3268	Methanol	1.67
0.1050	0.3267	Methanol/20 % Dioxane	3.25
0.1050	0.3267	Methanol/40 % Dioxane	5.67
0.1050	0.3267	Methanol/60 % Dioxane	8.36

values of k are recorded in column 6 of Table 16 and show fairly good agreement with the experimental values.

The methylation of sodium ethoxide was studied only in pure ethanol (Table 17). The rate constant was the same as that reported by Robertson and Acree [10]. The reaction is somewhat faster than that between methyl iodide and sodioacetoacetic ester without excess ester.

Table 18 shows that a second order rate constant is also obtained when methyl iodide reacts with an equilibrium mixture of two different salts.

The influence of added dioxane on the reaction between methyl iodide and sodium methoxide in methanol is shown in Tables 19–23. The relationship between the rate constant and the volume content of added dioxane is not linear in this case.

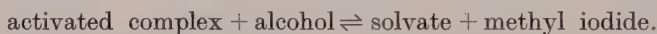
Discussion

The results obtained in the present investigation on the alkylation of sodioacetoacetic esters and in that by Pearson on the alkylation of sodiomalonic ester are qualitatively the same. In both cases the addition of free ester increases the rate of reaction. Pearson assumes that this is due to the solvolysis of the ester anions: The solvolysis is suppressed by the addition of ester. However, the possibility cannot be excluded that other effects are also operating. Malonic ester has a lower dielectric constant than methanol. Addition of ester will thus depress the dielectric constant

of the solution and this would increase the rate of the reaction between the ester anions and the alkyl halide. A depression of the dielectric constant would also diminish the ionization of the ester enolate and thus favour the reaction between the enolate molecules and the methyl iodide.

The investigation of Pearson is a good example of a critical treatment of competitive reactions. Another example of such reactions is that illustrated in Table 18 in the present paper. From both examples it follows that the interpretation of data for alkylation reactions must be done with caution.

In the interpretation of the reaction between alkyl halides and sodioacetoacetic esters it must be taken into account that the acidic properties of acetoacetic esters are much greater than that of malonic ester. According to Pearson and Dillon [14] the acid dissociation constant of malonic ester in water at 25° is 5×10^{-14} and that of acetoacetic ester 2.1×10^{-11} . The dissociation constant of methyl acetoacetate is surely not less than that of acetoacetic ester. The great difference makes it likely that the solvolysis of the acetoacetic ester anions can be neglected. The increase in the reaction rate caused by the addition of free ester can thus not depend on a depression of the solvolysis of the ester anions. Another reason is that the addition of dioxane, acetonitrile, and methyl acetoacetate causes almost the same increase in the rate of reaction. The addition of methyl acetoacetate would have a much greater influence, if the solvolysis was important. (Compare eq. (4), (5), and (9).) It thus seems that the most important factor is the depression of the alcohol concentration. A possible explanation of this circumstance is that the alcohol molecules compete with the methyl iodide in the activated complex:



A lowering of the alcohol concentration would thus favour the coordination of the methyl iodide.

It may be of interest here to note what Scatchard [15] says about the inhibiting effect of acetone and nitrobenzene on the hydrolysis of benzhydryl chloride: "The results of Farinacci and Hammett [16] for the solvolysis of benzhydryl chloride show that the dielectric constant is a very crude measure of the effect of solvents on this reaction and also indicate a very interesting effect of nitrobenzene and acetone, which may perhaps be explained by the highly polar, doubly bound oxygens competing with those of the hydroxyl groups for the positions where the latter can react."

The great accelerating effect of dioxane upon the reaction between sodium methoxide and methyl iodide in methanol might depend on alterations of the dielectric constant but also on the depression of the methanol concentration. Also in this reaction a competition between the alcohol and the methyl iodide in the activated complex can be assumed.

Summary

The reaction of methyl iodide with the sodium compounds of ethyl acetoacetate and methyl acetoacetate in the presence of free ester has been studied in ethanol and methanol, respectively. It was found that the free ester accelerated the reaction. Purely second order reactions were obtained. It can therefore be stated that the free esters are not appreciably alkylated. The addition of dioxane and acetonitrile had almost exactly the same influence upon the rate of reaction as the free ester. It

is thus likely that the increase of the reaction rate is due to the depression of the alcohol concentration, because the alcohol competes with the alkyl halide in the activated complex.

The reaction between methyl iodide and sodium methoxide in methanol-dioxane mixtures has also been investigated. The addition of dioxane had a great accelerating effect on the rate of reaction.

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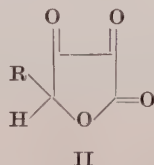
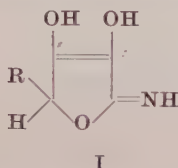
Tryckt den 29 mars 1956

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Über die Entstehung von *aci*-Reduktonen bei der Spaltung des 4-Aryl-2,3-diketo-butyrolaktone

Von HANS HASSELQUIST

Vor kurzer Zeit beschrieben H. DAHN und Mitarb. (1) eine interessante Gruppe von Reduktonen, die 4-Aryl-2-oxytetronimide. Dieselben gehören, wie Dahn angibt, und der Formel (I) entnommen werden kann, zu der Klasse der *aci*-Reduktone. Im Anschluss an die Arbeiten dieses Institutes über *aci*-Reduktone wurde das Oxydationsprodukt des obengenannten Tetronimides (II) hergestellt und die bei dessen Spaltung in Wasser auftretenden Substanzen wurden untersucht.



Es zeigte sich zunächst, dass die untersuchten Butyrolaktone, nämlich das 4-Phenyl-2,3-diketo-butyrolakton, das 4-*m*-Nitrophenyl-2,3-diketo-butyrolakton und das *p*-Phenyl-bis-2,3-diketo-butyrolakton in wässriger Lösung bei ca 70° unter CO₂-Entwicklung gespalten werden. Nach Beendigung dieser Reaktion enthielt die wässrige Lösung in allen Fällen ein *aci*-Redukton. Dies wurde durch die folgende Titration der erhitzten wässrigen Lösung des 4-Phenyl-2,3-diketo-butyrolaktone mittels Tillmans' Reagenz (TR) aufgezeigt.

Das nach DAHN und LAWENDEL (2) dargestellte Butyrolakton reduzierte nach der Erhitzung in wässriger Lösung und darauf folgendes Ansäuern mit HAc eine TR-Lösung stark.

Dabei wirkt das Natriumacetat als Katalysator der Decarboxylierung. Um diese Beobachtung sicherzustellen wurde die Decarboxylierung quantitativ bestimmt und die Lösung wurde während des Reaktionsverlaufes mit TR titriert um die maximale Endiol-Bildung zu ermitteln.

Versuch I: 9,8 mg 4-Phenyl-2,3-diketo-butyrolakton wurden in 10 ml Wasser aufgeschlämmt, mit 1 g NaAc versetzt und auf dem Wasserbad auf 70° erhitzt und mit 0,00896-*n*. TR-Lös. titriert. Der gesamte TR-Verbrauch betrug 2,25 ml. Wenn demgemäß ein Redukton mit *E* = 82 (Butyrolakton nach Verlust von 1 Mol CO₂) entsteht, so hat sich dieses zu 20 % der theor. möglichen Menge gebildet.

Versuch II: 5 g Butyrolakton wurden in 50 ml Wasser auf dem Wasserbad auf 80° erhitzt, wobei die CO₂-Entwicklung deutlich beobachtet werden konnte. Die Lösung wurde stetig gelber, nach einigen Minuten opalescent und schied während der Reak-

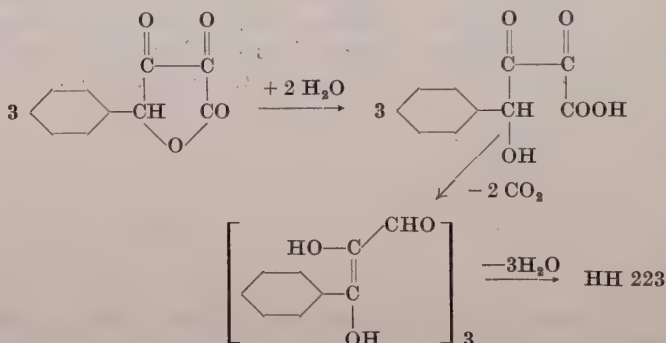
tion eine gelbbraune Fällung aus. Wenn die CO_2 -Entwicklung aufhörte (nach etwa 30 Min.) wurde die Fällung abfiltriert, mit etwas Wasser gewaschen und getrocknet. Der etwas klübbigen Fällung wurde durch Behandlung mit wenig Alkohol der klübbige Anteil entzogen, worauf intensiv gelbe Nadeln zurückblieben, die abfiltriert und mit wenig Alkohol vorsichtig gewaschen wurden; die Ausbeute an diesem Rohprodukt vom Schmp 218° betrug 100 mg.

Nach Umkristallisieren aus wenig Chloroform verblieb der Schmp der gelben Kristalle 218°. Sie sind schwerlöslich in Wasser, ziemlich leicht löslich in Alkohol und Chloroform.

Für HH 223 ($\text{C}_9\text{H}_6\text{O}_2$) ₃	Ber. C 73,96 %	H 4,14 %
	Gef. 74,14 %	4,15 %

Wie zu erwarten reduzierte diese Stbst. TR *nicht*.

Das Filtrat der gelben Nadeln wurde mit NaCl gesättigt, wodurch ein rotgelbes Öl ausfiel, das nicht zur Kristallisation gebracht werden konnte und grosse Mengen TR in saurer Lösung entfärbte.



Darstellung von *p*-Phenylen-bis-2,3-diketo-butyrolakton

a) Darstellung des Xylylendibromides

Zu 10,6 g (11,6 ml) *p*-Xylol, unter Rückfluss auf dem Sandbad zum Sieden erhitzt, liess man 32 g (10,8 ml) Brom aus einem Tropftrichter zufließen, der unter der Oberfläche der Flüssigkeit mündete, und zwar so langsam, dass keine Bromdämpfe entweichen sondern die Reaktion ruhig verlief. Wenn nach einer Reaktionszeit von etwa 90 Minuten alles Brom zugesetzt war wurde die Flüssigkeit noch weitere 5 Minuten erhitzt. Nach dem Erkalten erstarrte der Kolbeninhalt zu einer Kristallmasse von Xylylen-dibromid, die mit wenig Alkohol verrührt und dann abfiltriert wurde, wobei sich die Verunreinigungen leicht im Alkohol lösten.

Ausbeute an reiner Stbst.: 11 g vom Schmp 144°.

b) Darstellung des Terephthalaldehydes, nach WEGSCHEIDER (3)

11 g Xylylendibromid wurden in 110 ml einer wässrigen Lösung von 11 g Bleinitrat unter Rückfluss zum Sieden erhitzt. Nach einstündigem Kochen entwichen Stickoxyde aus dem in der Reaktionsmischung befindlichen Öl. Nach etwa 6 Stunden wurde keine Stickoxyd-Entwicklung mehr beobachtet. Aus der warm filtrierten Lösung kristallisierte der Terephthalaldehyd sowie ein wenig Terephthalsäure aus. Der Rest wurde aus dem Filtrat mit heissem Wasser extrahiert.

Die Kristallfraktionen wurden nun zusammen in Chloroform gelöst, während die Terephthalsäure zurückblieb, durch Einengen der Chloroformlösung wurden 2 g Terephthaldialdehyd vom Schmp 110–112° gewonnen. Aus Äther umkristallisiert; Schmp 116°C.

c) *Darstellung von p-Phenylen-bis-oxytetronimid nach DAHN (4)*

2 g Terephthaldialdehyd wurden in 4 ml Dioxan gelöst und im Stickstoffstrom bei Zimmertemperatur zu einer 2-n. K_2CO_3 -Lösung zugegeben, die 21,2 g Glyoxal-bisulfit und 4,24 g KCN enthielt. Nach 60 Minuten wurde das Reaktionsgemisch durch Zusatz von Essigsäure auf pH 7 gebracht. Dadurch wurde die Lösung opalescent und schied allmählich ein weisses mikrokristallinisches Pulver aus. Nach weiterem zweistündigem Stehen (pH 7 kontrolliert) wurde die Fällung abfiltriert, mit Wasser gewaschen und getrocknet. Die Ausbeute an p-Phenylen-bis-oxytetronimid betrug 1,5 g.

d) *Darstellung des p-Phenylen-bis-2,3-diketo-butyrolaktones*

1,5 g p-Phenylen-bis-diketo-tetronimides wurden in 12,5 ml einer 2-n. Schwefelsäure unter Zusatz von 5 ml Aceton aufgeschlämmt, worauf das Reaktionsgemisch auf 0° abgekühlt und dann tropfenweise mit 15 ml einer 10%igen Lösung von Natriumnitrit versetzt wurde. Unter NO-Entwicklung ging die Ausgangssubstanz nach dem letzten Zusatz des Nitrites allmählich in Lösung. Während weiterem dreistündigem Stehen bei Zimmertemperatur fiel das Oxydationsprodukt in Form farbloser, glänzender Kristalle aus, die mit wenig Wasser gewaschen und getrocknet wurden. Ausbeute: 1,4 g. Die Substanz schmilzt über 300°, färbt sich braun bei etwa 120°.

Beim Aufkochen der Substanz mit Wasser trat eine deutliche Entwicklung von CO_2 ein und die Lösung zeigte nun im sauren Gebiet kräftige Reduktion des TR.

			C	H
Für HH 228	$C_{14}H_6O_8 \cdot 6 H_2O$	Ber.	40,98 %	4,42 %
		Gef.	41,11 %	4,80 %

e) *Titration des mit Wasser erhitzten p-Phenylen-bis-2,3-diketo-butyrolaktones*

13,0 mg Butyrolakton wurden in 10 ml Wasser aufgeschlämmt, nach Zusatz von 1 g NaAc auf dem Wasserbad bis 70° erwärmt und mit einer 0,00896-n. TR-Lös. titriert. Der Gesamtverbrauch betrug 7,35 ml. Wenn sich also ein Endiol von E = 63 gebildet hat (das Butyrolakton, das unter Bildung von Bis-Endiol 2 CO_2 abgespalten hat) so entspricht dies dem Verbrauch von 52 % des theoretischen Wertes an Bis-endiol.

f) *Versuch zur Isolierung des gebildeten aci-Endioles*

0,5 g Bis-diketo-butyrolaktones, in 10 ml Wasser aufgeschlämmt und mit 2 g NaAc versetzt, wurden auf dem Wasserbad vorsichtig erwärmt bis CO_2 -Entwicklung einsetzte. Bei 20° setzte die CO_2 -Entwicklung durch die katalytische Einwirkung von NaAc fort. Nach 5 Stunden wurde die Reaktionsmischung mit Kochsalz gesättigt, mit Äthylacetat dreimal ausgeschüttelt und mit wasserfreiem Natriumsulfat getrocknet, sodann im Vacuum bei 20° zur Trockne verdampft.

Der Rückstand, ein gelbes Öl, reduzierte TR in saurer Lösung stark. Auf Zusatz von einigen ml Äthylacetat fiel ein mikrokristallinisches Pulver aus, das abfiltriert und mit Äthylacetat gewaschen wurde. Die Substanz wird mit HH 229, das Filtrat mit F bezeichnet.

Die Sbst. 229 (Ausbeute 45 mg vom Schmp 300°) verbraucht TR in saurer Lösung (vgl. im übrigen die Titration).

Die Sbst. 229 ist schwerlöslich in Äthylacetat, etwas löslich in siedendem Wasser, woraus sie beim Erkalten in Form kleiner farbloser Prismen auskristallisiert. (Die Fluoreszenz stammt möglicherweise von einer Verunreinigung.)

g) *Titration der Sbst. 229 mit TR*

9 mg Sbst. wurden in 10 ml Wasser gelöst; davon wurde 1,0 ml mit einer 0,00896-*n*-TR-Lösung titriert. 0,9 mg Sbst. verbraucht dabei 1,42 ml.

$$E_{\text{gef.}} = \frac{0,9}{1,42 \cdot 0,00896} = 71$$

$$\text{C}_{12}\text{H}_{10}\text{O}_6 \quad E_{\text{ber.}} = 63.$$

h) *Untersuchung des Filtrates F*

Das Filtrat wurde wiederum im Vacuum zur Trockne verdampft, aufs neue mit etwas Äthylacetat verdünnt, wodurch das gelbe Öl leicht in Lösung ging und die weitere Menge von 10 mg zurückblieb, die abfiltriert wurde. Das Filtrat wurde eingedunstet. Aus dem so erhaltenen gelben Öl kristallisierten kleine Stäbchen aus, die wegen der übereinstimmenden Löslichkeit von Kristallen und Öl noch nicht isoliert werden konnten.

Öl + Sbst. verbrauchten in saurer Lösung kräftig TR

i) *Papierchromatographische Versuche mit Sbst. 229 und Filtrat F*

Lösung: *n*-Butanol-gesättigtes Wasser. — Papier: Munktel OB. Entwickler: TR gelöst in Alkohol (0,005-*n*).

Die Sbst. 229 ergab $R_f = 0,84$. Das Öl wanderte ganz mit der Front und ergab deshalb bei der Entwicklung eine horizontale weisse Linie bei der Entwicklung ($R_f = 1,0$).

Die mit *p*-Phenyl-bis-2,3-diketo-butyrolakton ausgeführten Versuche zeigen, dass sich bei der Erhitzung mit Wasser unter NaAc-Zusatz (aber auch ohne Zusatz) zwei verschiedene ac-Reduktone bilden, von denen das eine in reiner Form isoliert werden konnte.

Darstellung von 4-(*m*-nitro-phenyl)-2,3-diketo-butyrolakton.

5 g 4-(*m*-nitro-phenyl)-2-oxy-tetronimid wurde nach DAHN u. Mitarb. in 53 ml 2-*n*. H_2SO_4 aufgeschlämmt und mit 20 ml Aceton und 29,6 ml 10 %iger Natriumnitritlösung) unter Eiskühlung tropfenweise versetzt. Nach zweistündigem Stehen bei Zimmertemperatur wurde das Aceton abgedunstet, worauf die rückständige klare Lösung in den Kälteschrank zur Kristallisation gestellt wurde. Es resultierten kleine, bräunliche Nadeln in kugelrunden Aggregate, die mit etwas Wasser gewaschen wurden. Die Ausbeute an Rohprodukt betrug 1,7 g, die bei 128–130° unter Gasentwicklung schmolzen. (HH 230.)

			C	H	N
Für HH 230	$\text{C}_{10}\text{H}_5\text{O}_6\text{N} \cdot 3 \text{H}_2\text{O}$	Ber.	41,53 %	3,83 %	4,84 %
		Gef.	42,22 %	4,09 %	5,14 %

Auch dieses Lakton lieferte bei Erhitzen mit Wasser ein Redukton. Durch Eindunsten der wässrigen Lösung konnte bis jetzt noch kein Produkt isoliert werden.

Titration des mit Wasser erhitzten 4-(*m*-nitro-phenyl)-2,3-diketo-butyrolaktons mit TR

8,7 mg Sbst. 230 wurden in 10 ml Wasser mit 1 g NaAc versetzt auf dem Wasserbad bis 70° erwärmt und mit 0,00896-*n*. TR-Lös. titriert, wobei 4,5 ml verbraucht wurden. Dieser Verbrauch würde der Bildung von rund 67 % Endiol vom *E* = 105 entsprechen. Butyrolakton minus 1 Mol CO₂.

Versuch zur Darstellung von 5-*p*-phenylen-bis-3,4-dioxytetrone

100 mg *p*-Phenylen-bis-2,3-diketo-butyrolakton wurden im Aghatmörser fein pulverisiert und in 5 ml Aceton + 5 ml Amylalkohol aufgeschlämmt. Die Lösung wurde mit H₂S gesättigt und unter Verschluss 3 Tage bei Zimmertemperatur aufbewahrt. In dieser Zeit verwandelte sich das ungelöste Pulver in eine gutkristallisierte Substanz, die abfiltriert, mit Aceton gewaschen und getrocknet wurde. Die Ausbeute an diesem Rohprodukt war quantitativ. Schmp 300°. Durch Umkristallisieren aus siedendem Wasser erhielt man kleine, farblose gut ausgebildete Tafeln. Subst. 231. Dieselbe verbraucht TR in saurer Lösung.

Titration mit TR.

1,9 mg Sbst. 231 in ca 10 ml Wasser gelöst wurde direkt mit einer 0,00896-*n*. TR-Lös. titriert.

$$E_{\text{gef.}} = \frac{1,9}{3,35 \cdot 0,00896} = 64 \quad E_{\text{ber.}} \text{ für } C_{14}H_{10}O_8 \cdot 2 H_2O = 86$$

			C	H
Für HH 231	C ₁₄ H ₁₀ O ₈ · 2 H ₂ O	Ber.	49,13 %	4,12 %
		Gef.	49,48 %	4,51 %

Ich danke herzlichst Herrn Prof. H. von EULER für sein wohlwollendes Interesse an dieser Arbeit und Herrn Ingenieur ERIKSSON für eifrige Hilfe bei der Ausführung der Versuche.

Universität Stockholm, Institut für organ.-chemische Forschung.

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Uppsala 1956. Almqvist & Wiksells Boktryckeri AB



The formation of an ether-soluble complex between phosphatide and histamine

By KERSTIN M. LINDAHL

It has been claimed by Euler (1) that lecithin combines with adrenaline and nor-adrenaline to form an ether-soluble compound. These findings were more fully investigated by Norlander (2). The authors discuss the physiological importance of these compounds with regard to their possible role as a storage compound of adrenergic transmitter substances.

In earlier work it was found that lecithin can diminish the histamine induced contractions of isolated guinea pig ileum. It was therefore felt of interest to investigate the possibility of this effect being due to a complex formation between phosphatide and histamine.

Experimental procedure

Samples of lecithin prepared according to Pangborn (3) were dissolved in diethyl ether and gently shaken with the same volume of an aqueous solution of histamine in buffer. When the mixture had separated in two phases half of the water phase was drawn off from the bottom and neutralized, if necessary. The small amounts of dissolved ether were removed by passing a current of air through the samples. The amount of histamine remaining in the water phase was then assayed on an isolated piece of guinea pig ileum. The ether phase was shaken with a salt solution containing salts in the proportion of Tyrodes solution but twice the concentration; NaHCO_3 was omitted from the medium. The water phase was drawn off, diluted with an equal quantity of NaHCO_3 (0.2 %) and its histamine content assayed.

In all experiments controls were treated in the same way as the samples: the buffer solution without histamine was treated with the ether-phosphatide solution and a sample of histamine buffer was treated with ether. After separation of the two phases in the controls, the pooled water and ether-phases were treated in the same way as the samples.

Samples of cephalin prepared according to Folch (4) were also used for the same type of experiments.

Table 1. Histamine conc. 2 $\mu\text{g}/\text{ml}$ in 0.2 % NaHCO_3 .

Phosphatide concentr. in ether %	Histamine, left in buffer after treatment %	Histamine recovered from ether %	Total recovery %
Lecithin 8	56	30	86
"	72	33	105
"	60	lost	—
"	60	28	88
"	56	28	84
Cephalin 0.9	64	22	86
"	58	20	78
"	60	22	82
"	60	22	82
"	57	20	77

Table 2. Histamine conc. 1 $\mu\text{g}/\text{ml}$; lecithin conc. 45 mg/ml .

Buffer	Carbonate 0.5M bicarbonate 0.1M	Prim. phosph. 0.05M NaOH 0.2M	Citrate 0.1M NaOH 0.1M
pH	9.7	8.0 7.8 7.5 6.9	5.9 4.0
Histamine, left after treat- ment, %	12	25 40 40 50	90 85

Table 3. Histamine conc. 3 $\mu\text{g}/\text{ml}$, cephalin conc. 10 mg/ml , Borax 0.05M, NaH_2PO_4 0.05M.

ph	9.0	8.4	8.0	7.4	7.0	6.4	5.8
Histamine, left after treat- ment, %	35	37	40	58	65	70	80
Histamine, recovered from ether, %	60	62	41	21	14	8	9

Results

In Table 1 the results of some experiments at constant pH are presented, showing the reproducibility of the complex formation. The individual variations do not exceed the experimental error for the bioassay.

In tables 2 and 3 the influence of the pH in the water phase is shown.

Discussion

The experimental results indicate that histamine forms some sort of complex with lecithin and cephalin and thereby becomes ether-soluble. This finding has to be considered when histamine is to be quantitatively extracted from phospholipid rich tissues.

The pH of the water phase has a great influence on the complexing of phosphatide and histamine. This suggests that the free phosphoric-acid group of the phosphatidyllic acid plays an important role in the complex formation.

A discussion of the possible physiological significance of these findings seems unprofitable until more data about the complex are obtained.

This work was partly carried out at the Pharmacological Department, the University of Edinburgh, on a Florence Quensel's scholarship.

I am indebted to Prof. P. E. Lindahl for valuable discussion and to Prof. J. H. Gaddum for the privilege of carrying out part of this work in his laboratory and for the kind interest he has shown in it.

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Verhalten der Ascorbinsäure in Yoshida-Ascites-Sarkom-Ratten bei deren Behandlung mit Deacetylcolchicin allein und zusammen mit Hydrocortison und ACTH

Von LUIGI RESEGOTTI

Die katalatische Wirkung der Leber und Milz von Ratten ist in Gegenwart von Yoshida-Ascites-Sarkom (wie bekanntlich auch anderer Tumoren) erniedrigt. Wie in einer vorhergehenden Arbeit in diesem Arkiv mitgeteilt wurde (1), wird die katalatische Wirkung der Ascites-Tumor-Lebern durch Injektion von *Deacetylcolchicin* (DAC) (bzw. dessen Tartrat) auf ihren normalen Wert zurückgeführt, während dieser Effekt durch *Colchicin selbst nicht eintritt* (2). Dieser Befund hat die Hypothese veranlasst, dass das Deacetylcolchicin dem nach NAKAHARA (3) in Tumorlebern auftretenden Toxohormon entgegenwirkt.

Zur Verfolgung dieser Hypothese wird untersucht, welche Änderungen in der Zusammensetzung und besonders hinsichtlich der Wirkstoffe in den Tumorlebern durch Deacetylcolchicin-Injektionen in die Ratten eintreten.

In der vorliegenden Mitteilung wird zunächst der Einfluss des Deacetylcolchicins auf den *Ascorbinsäure-Gehalt* der Ascites-Sarkom-Lebern studiert.

Die bedeutende Rolle, welche dem C-Vitamin (Ascorbinsäure, AS) in den oxydo-reduktiven Systemen des Tierkörpers zukommt, hat die Vermutung erregt, dass AS in den rasch wachsenden Tumorgeweben in hoher Konzentration vorhanden sei. Tatsächlich fand WOODWARD (4) schon 1935 im Sarkom Philadelphia Nr. 1 einen höheren Ascorbinsäure-Gehalt als in anderen Geweben. Wenn sich die Resultate von WOODWARD auch nur auf wenige Fälle beziehen und hauptsächlich zur Darlegung einer Bestimmungsmethode, weniger als selbstständiges Versuchsergebnis angegeben wurden, so waren sie immerhin signifikativ. Der Gehalt der Ascorbinsäure in der Leber einer tumortragenden Ratte erschien gegenüber demjenigen der normalen Rattenleber deutlich erniedrigt, während in Milz und Niere der entsprechende Unterschied geringer war. Ascorbinsäure verhält sich in dieser Hinsicht wie Glutathion.

WOODWARDS Angaben wurden kürzlich von SHIBATA (5) bestätigt. Der japanische Autor, der mit Yoshida-Ascites-Tumor arbeitete, beobachtete, dass im Tumor und im Ascites der Ascorbinsäure-Gehalt während der Entwicklung des Tumors stieg, und zwar bis zur Erreichung der Werte 59,7 mg% bzw. 18,7 mg%. Gleichzeitig nahm AS in den Organen ab.

Es erscheint von Interesse zu erforschen, in welcher Weise der von einem cytotastischen Stoff ausgeübte Effekt sich im tierischen und pflanzlichen oxydativen Stoffwechsel des Systems AS-Glutathion äussert. Diese Untersuchung wurde am gleichen Material parallel mit einer Arbeit über *Glutathion* ausgeführt (RESEGOTTI u. EULER). Die Absicht war u. a., den Einfluss aufzuklären, welchen die Assoziation eines Colchicin-Derivates mit der antitumoralen Wirkung einer kürzlich in die Therapie eingeführten Substanz, dem Deacetylcolchicin (DAC), mit Hydrocortison und mit ACTH ausübt. Auch von diesem Gesichtspunkt aus konnte die Ascorbinsäure sich als Test-Substanz erweisen.

Es wurden die Arbeiten von COSENTINI (7), COLIZZI, ROSSANDA und VACCARI (6) berücksichtigt, nach denen ACTH und Cortison auf den Ascorbinsäure-Gehalt der Rattenorgane keinen Einfluss ausüben. Wenn man indessen einen Unterschied beobachten konnte zwischen tumortragenden Ratten, welche nur mit DAC oder nur mit ACTH und Hydrocortison behandelt waren, und denjenigen, welche die genannten Stoffe gleichzeitig erhielten, so würde dieses Ergebnis zu Gunsten der Annahme sprechen, dass ein Synergismus zwischen den Wirkungen von ACTH und Cortison und DAC besteht.

Versuche:

Gehalt an Ascorbinsäure (AS)

Hinsichtlich des Versuchsmaterials und der Methodik wird auf eine folgende Arbeit über *Glutathion*-Gehalt nach DAC-Eingaben verwiesen. Die Ascorbinsäure wurde hier stets mittels Dichlorphenol-indophenol bestimmt, und zwar in den mit 2%iger Sulfosalicylsäure behandelten Organ- bzw. Gewebe-Extrakten.

Gewicht bzw. Nummer der Ratte	Organ	Gewicht g	mg AS/100 g des Organs	Gesamtmenge AS	mg AS/100 g d. Tiergew.
<i>A. Normale Ratten</i>					
130 g	Leber	6,8	22,5	1,53	1,17
	Milz	0,466	42,8	0,199	0,153
	Niere	1,236	14,4	0,177	0,136
	Nebenniere	0,029		0,145	0,111
135 g	Leber	6,615	24	1,58	1,17
	Milz	0,498	25	0,124	0,095
	Niere	1,14	11,2	0,127	0,094
	Nebenniere	0,025	598	0,149	0,110
120 g	Leber	7,33	36	2,63	2,19
	Milz		32,1	0,149	0,124
	Niere		10,2	0,121	0,101
	Nebenniere		460	0,110	0,092
90 g	Leber	5,079	32	1,62	1,80
	Milz	0,362	34	0,123	0,136
	Niere	0,785	11,2	0,087	0,097
	Nebenniere	0,022	508	0,111	0,124

Versuche (Forts.)

Gewicht bzw. Nummer der Ratte	Organ	Gewicht g	mg AS/100 g des Organs	Gesamtmenge AS	mg AS/100 g d. Tiergew.
<i>B. Normale Ratten, injiz. mit DAC</i>					
140 g	Leber	6,733	34	2,28	1,655
	Milz	0,49	34	0,181	0,129
	Niere		18,8	0,200	0,143
	Nebenniere		595	0,172	0,123
132 g	Leber	6,655	32	2,100	1,59
	Milz	0,420	44	0,184	0,14
	Niere	1,080	22	0,237	0,18
	Nebenniere	0,031	515	0,159	0,12
<i>C. Normale Ratten, injiz. mit Hydrocortison</i>					
130 g	Leber	6,600	33	2,178	1,67
	Milz	0,615	48	0,295	0,227
	Niere	1,060	16,4	0,173	0,133
	Nebenniere	0,019	572	0,108	0,083
140 g	Leber	7,480	33	2,468	1,76
	Milz	0,445	55	0,244	0,174
	Niere	1,170	21	0,245	0,171
	Nebenniere	0,027	561	0,151	0,108
<i>D. Ratten mit Ascites</i>					
122 g	Leber	7,33	13	0,952	0,78
	Milz	0,502	—	—	—
	Niere	1,335	16,6	0,201	0,181
	Nebenniere	0,038	262	0,099	0,081
	Ascites	—	—	—	—
	Tumor	—	18,8	—	—
135 g	Leber	8,307	12,5	1,058	0,76
	Milz	0,775	18,8	0,145	0,108
	Niere	1,305	9,25	0,121	0,089
	Nebenniere	0,030	250	0,075	0,054
	Ascites	—	10,5	—	—
	Tumor	—	20	—	—
134 g	Leber	8,500	20	1,7	1,27
	Milz	0,686	7,3	0,05	0,037
	Niere	1,180	10,5	0,123	0,092
	Nebenniere	0,032	343	0,109	0,081
	Ascites	—	11,5	—	—
	Tumor	—	28	—	—
Nr. 5	Leber	8,0707	26		
	Milz	0,513	33,9		
	Niere	1,100	11,2		
	Nebenniere	0,023	600		
	Ascites	—	15		
	Tumor	—	42		

Versuche (Forts.)

Gewicht bzw. Nummer der Ratte	Organ	Gewicht g	mg AS/100 g des Organs	Gesamtmenge AS	mg AS/100 g d. Tiergew.
Nr. 6	Leber	9,53	23		
	Milz	0,556	33,3		
	Niere	1,283	14,4		
	Nebenniere	0,032	377		
	Ascites	—	13		
	Tumor	—	32		
Nr. 7	Leber	8,332	20		
	Milz	0,675	30,2		
	Niere	1,210	8,8		
	Nebenniere	0,032	280		
	Ascites	—	13		
	Tumor	—	34		
Nr. 8	Leber	7,85	22,5		
	Milz	0,894	26		
	Niere	1,307	10,8		
	Nebenniere	0,030	280		
	Ascites	—	10,8		
	Tumor	—	34		
Nr. 9	Leber	8,450	26		
	Milz	0,496	35		
	Niere	0,910	13,7		
	Nebenniere	0,029	420		
	Ascites	—	11		
	Tumor	—	32		
Nr. 10	Leber	6,14	20		
	Milz	0,552	36		
	Niere	1,014	11		
	Nebenniere	0,029	304		
	Ascites	—	11		
	Tumor	—	—		
Nr. 11	Leber	6,82	20		
	Milz	0,933	37		
	Niere	0,830	8,6		
	Nebenniere	0,022	209		
Nr 12, 80 g	Leber	4,950	21	1,039	1,179
	Milz	0,340	32	0,108	0,136
	Niere	0,908	10,5	0,095	0,119
	Nebenniere	0,021	284	0,059	0,004
	Ascites	—	—	—	—
	Tumor	—	42,5	—	—
Nr. 13, 128 g	Leber	8,245	28	2,308	1,800
	Milz	0,490	42	0,205	0,160
	Niere	1,123	12	0,134	0,105
	Nebenniere	0,031	296	0,091	0,071
	Ascites	—	—	—	—
	Tumor	—	40	—	—

Versuche (Forts.)

Gewicht bzw. Nummer der Ratte	Organ	Gewicht g	mg AS/100 g des Organs	Gesamtmenge AS	mg AS/100 g d. Tiergew.
<i>E. Ascites + DAC</i>					
<i>Tiere, einen Tag nach der Injektion getötet</i>					
128 g	Leber	7,078	26,5	1,873	1,441
	Milz	0,460	17,2	0,078	0,060
	Niere	1,012	6,25	0,063	0,048
	Nebenniere	0,028	516	0,149	0,115
160 g	Leber	9,540	32	3,052	1,926
	Milz	0,720	36	0,259	0,162
	Niere	1,836	16,4	0,300	0,187
	Nebenniere	0,042	510	0,214	0,133
<i>F. Tiere, drei Tage nach der Injektion getötet</i>					
134 g	Leber	7,286	18,5	1,347	1,005
	Milz	0,690	21	0,144	0,108
	Niere	0,988	9	0,088	0,066
	Nebenniere	0,025	400	0,1	0,074
	Ascites	—	42	—	—
	Tumor	—	31	—	—
142 g	Leber	6,074	20	1,214	0,855
	Milz	0,836	18,6	0,155	0,109
	Niere	0,870	12,5	0,108	0,076
	Nebenniere	0,030	431	0,129	0,091
	Ascites	—	12,5	—	—
	Tumor	—	36	—	—
150 g	Leber	6,542	17,5	1,144	0,763
	Milz	0,672	28,8	0,193	0,129
	Niere	1,200	13,1	0,157	0,104
	Nebenniere	0,031	772	0,239	0,159
	Ascites	—	16	—	—
	Tumor	—	36,7	—	—
<i>G. Ascites + Hydrocortison</i>					
145 g	Leber	6,500	20	1,3	0,89
	Milz	0,852	20,3	0,170	0,119
	Niere	1,097	11,2	0,120	0,083
	Nebenniere	0,022	499	0,109	0,075
	Ascites	—	18	—	—
	Tumor	—	32	—	—
154 g	Leber	7,080	20	1,416	0,919
	Milz	0,420	30	0,126	0,086
	Niere	0,990	11	0,108	0,070
	Nebenniere	0,028	444	0,124	0,080
	Ascites	—	13,5	—	—
	Tumor	—	27,5	—	—

Versuche (Forts.)

Gewicht bzw. Nummer der Ratte	Organ	Gewicht g	mg AS/100 g des Organs	Gesamtmenge AS	mg AS/100 g d. Tiergew.
128 g	Leber	6,230	30	1,869	1,44
	Milz	0,575	35	0,201	0,157
	Niere	0,940	16,4	0,153	0,119
	Nebenniere	0,029	584	0,169	0,132
	Ascites	16	—	—	—
	Tumor	39	—	—	—
<i>H. Ascites + ACTH</i>					
150 g	Leber	7,100	24	1,704	1,136
	Milz	0,660	40	0,264	0,176
	Niere	1,108	12	0,132	0,088
	Nebenniere	0,036	420	1,151	1,008
	Ascites	—	15	—	—
	Tumor	—	32	—	—
<i>I. Ascites + DAC + Hydrocortison</i>					
142 g	Leber	6,656	18	1,198	0,843
	Milz	0,967	25,5	0,246	0,173
	Niere	0,960	10,2	0,097	0,067
	Nebenniere	0,032	498	0,159	0,112
	Ascites	—	17	—	—
	Tumor	—	34	—	—
140 g	Leber	7,460	24	1,790	1,28
	Milz	0,950	23,4	0,222	0,158
	Niere	1,025	15,3	0,154	0,110
	Nebenniere	0,029	602	0,174	0,124
	Ascites	—	15	—	—
	Tumor	—	47,5	—	—
190 g	Leber	7,780	25	1,967	1,329
	Milz	0,724	41,4	0,299	0,202
	Niere	0,980	18,8	0,184	0,124
	Nebenniere	0,027	499	0,134	0,091
	Ascites	—	19	—	—
	Tumor	—	47,6	—	—
<i>K. Ascites + DAC + ACTH</i>					
190 g	Leber	10,987	23	2,327	1,224
	Milz	0,780	28	0,218	0,114
	Niere	1,395	14	0,195	0,102
	Nebenniere	0,044	440	0,193	0,101
	Ascites	—	17,5	—	—
	Tumor	—	23	—	—
180 g	Leber	9,675	26	2,515	1,397
	Milz	1,270	30	0,381	0,212
	Niere	1,265	12	0,151	0,084
	Nebenniere	0,050	400	0,2	0,111
	Ascites	—	6	—	—
	Tumor	—	31	—	—

Tabelle der Mittelwerte.

Ascorbinsäure in mg/100 g der frischen Organe.

Gruppe	Anzahl der Fälle	Leber	Milz	Niere	Nebenniere	Ascites	Tumor
Normal	4	28,6	33,5	11,7	517		
Normal + DAC	2	33	40,5	20,4	555		
Normal + Hydrocort.	2	33	51,5	18,7	566		
Ascites	13	21,4	29	11,7	313	12	32,5
Ascites + DAC	3	18,7	22,8	11,5	534	23,3	34,5
Ascites + Hydrocort.	3	23,3	28,4	14,8	509	15,8	32,9
Ascites + ACTH	1	24	40	12	420	15	32
Ascites + DAC + Hydrocort.	3	22,6	30,1	14,8	533	17	43
Ascites + DAC + ACTH	2	24,5	29	13	420	11,5	27

Ascorbinsäure mg/100 g des Gewichtes der Tiere.

Gruppe	Anzahl der Fälle	Leber	Milz	Niere	Nebenniere
Normal	4	1,566	0,124	0,107	0,109
Normal + DAC	2	1,612	0,134	0,161	0,121
Normal + Hydrocort.	2	1,71	0,200	0,152	0,095
Ascites	13	1,28	0,110	0,101	0,072
Ascites + DAC	3	0,87	0,115	0,082	0,108
Ascites + Hydrocort.	3	1,087	0,121	0,091	0,096
Ascites + ACTH	1	1,136	0,176	0,088	0,100
Ascites + DAC + Hydrocort.	3	1,15	0,178	0,100	0,109
Ascites + DAC + ACTH	2	1,31	0,163	0,093	0,106

Die Dosen der injizierten Substanzen per kg Tier waren: DAC 10 mg; Hydrocortison 5 mg; ACTH 10 mg.

Aus den obigen Tabellen ergeben sich folgende Tatsachen:

In Übereinstimmung mit SHIBATA wurde eine durch den Tumor hervorgerufene Erniedrigung des AS-Gehaltes der Leber festgestellt.

Keine oder fast keine AS-Erniedrigung tritt ein in den Nieren und in der Milz, dagegen ist sie deutlich in der Leber und in den Nebennieren. In letzterem Organ beträgt die Erniedrigung 35 % des Normalwertes und durch die erhebliche Zahl der Bestimmungen ist das Ergebnis in gewissem Grad signifikativ. Die AS-Erniedrigung in der Leber beträgt etwa 20 %.

Der Unterschied gegenüber den Daten von WOODWARD, der eine AS-Erniedrigung von 50 % fand, kann darauf beruhen, dass in unseren Versuchen die Zeit zwischen der Einimpfung des Tumors und der Tötung des Tieres kurz war.

Die Erniedrigung des AS-gehaltes in den Nebenniere konnte aufgehoben werden durch Behandlung des Tieres mit DAC und Hydrocortison. Die Assoziationen der Hormone mit DAC erwies sich nicht aktiver als die einzelnen Substanzen. Die Behandlung mit DAC war nicht fähig, die AS-Erniedrigung in der Leber zu hemmen.

Besprechung und Schlussfolgerung

Die Bedeutung wie auch der Mechanismus der Abnahme des AS-Gehaltes in den Organen tumortragender Tiere sind noch unklar. Man kann zur Erklärung zwei Annahmen heranziehen:

1. Dass die Entwicklung des Tumors selbst einen Teil der AS erfordert.
2. Dass eine vom Tumor erzeugte Substanz eine Giftwirkung ausübt.

Zu Gunsten der ersteren Annahme spricht die Tatsache, dass der AS-Gehalt im Tumorgewebe erhöht ist und zwar mit dem Wachstum des Tumors steigt, während man in den Parallelversuchen eine Erniedrigung des AS-Gehaltes der Organe beobachtet.

In den behandelten Tieren hat man indessen keine Parallelität beobachtet zwischen der Hemmung des Tumors und dem AS-Gehalt im Tumorgewebe oder in den Organen.

Die AS-Konzentration im Ascites wird durch die Behandlung nicht erniedrigt, sondern erscheint im Gegenteil vermehrt, und daher ist der Gehalt der Nebennieren auf den Normalwert zurückgeführt.

Andererseits war es von Interesse zu beobachten, dass sich bei stark entwickeltem Tumor und reichlichem Ascites in den nur mit Hormonen oder nur mit DAC behandelten Tieren keine Anzeichen von schlechtem Zustand erkennen liessen.

Zur Aufklärung dieses Phänomens scheint die Annahme einer Giftwirkung geeigneter. Wie wir schon bezüglich der Katalase hervorgehoben hatten, für welche man ähnliches Verhalten beobachtete, kann man annehmen, dass der Tumor toxische Stoffe erzeugt welche fähig sind, einerseits die Konzentrationen der Leberkatalase und andererseits die Konzentration von AS in den Nebennieren zu erniedrigen.

Das Deacetylcolchicin und die Hormone wirken in diesem Mechanismus zusammen indem sie die toxischen Wirkungen blockieren und die in unseren klinischen Arbeiten erwähnten subjektiven Empfindungen des Wohlbefindens hervorrufen.

Zusammenfassung

Der Ascorbinsäure-Gehalt der Leber, Milz, Nieren, Nebennieren, ferner des Ascites und des Tumorgewebes von Yoshida-Ascites-Sarkom-tragenden Ratten wurde untersucht und zwar während bzw. nach der Behandlung der Versuchstiere mit Deacetylcolchicin a) allein, und b) gleichzeitig mit Hydrocortison und ACTH.

In den mit Yoshida-Ascites-Tumoren injizierten Ratten wurde eine Erniedrigung des Ascorbinsäure-Gehaltes in allen Organen, besonders in den Nebennieren, festgestellt.

Die Normalwerte des Ascorbinsäure-Gehaltes werden erreicht teils mit Deacetylcolchicin (DAC) teils mit den Hormonen Hydrocortison und ACTH allein oder zusammen mit DAC.

Der Mechanismus der AS-Erniedrigung durch den Tumor wird besprochen und zur Erklärung des studierten von DAC, Hydrocortison und ACTH ausgeübten Hemmungseffektes eine Hypothese aufgestellt.

Riassunto

È stato studiato il comportamento di acido ascorbico del fegato, milza, rene, surrene, ascite e tumore solido in ratti portatori di tumori ascite di Yoshida durante il trattamento con deacetilcolchicina da sola od associata a idrocortisone e ACTH.

Negli animali iniettati con tumore si è osservato una diminuzione di vitamina C in tutti gli organi e in particolare nel surrene.

La normalizzazione dei valori si ottiene sia con la DAC che con gli ormoni da soli od associati al citostatico, non ostante che il tumore sia scarsamente inibito nella crescita dalla sostanza usate isolatamente, mentre le associazioni si mostrano più effective sotto questo aspetto.

Si discute il meccanismo di depressione dell'acido ascorbico da parte del tumore e si avanza una ipotesi per spiegare l'effetto protettivo esercitato dalla DAC, dall'ACTH e dall'idrocortisone.

Der Verfasser, *Istituto di Patologia Medica, Università di Torino*, hat die vorliegende Untersuchung am Institut für organ.-chemische Forschung der Universität Stockholm mit einem Stipendium von *Svenska Institutet* ausgeführt.

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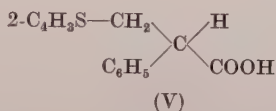
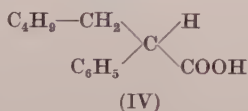
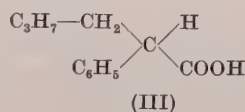
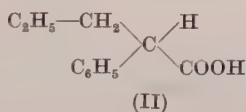
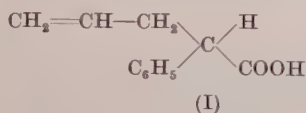


Configurational studies in the α -phenyl carboxylic acid series

By KURT PETTERSSON

With 9 figures in the text

In a series of papers (1-3) the steric connection between some α,β -diarylpropionic acids has been elucidated. Among these acids 2-thenylphenylacetic acid (V) offers a special interest, since this acid constitutes the end point of a chain of substances by means of which the configurations of the diarylpropionic acids are assigned a place in the system of D-glyceraldehyde. The remaining members of the chain are allylphenylacetic acid (I), sterically related to glyceraldehyde by Fredga and Westman (4), α -phenylvaleric (II), α -phenylcaproic (III) and α -phenyloenantic (IV) acids.



(+)-Allylphenylacetic acid (I) has been catalytically hydrogenated to (+)-(II) with retention of configuration, thereby establishing the steric relation between the two acids. The acids (II), (III), and (IV) have been sterically connected by means of conventional methods (*vide infra*).

In 1953 Fredga (5) reported a method for the steric correlation of aromatic and aliphatic compounds, including ring fission of optically active 2-thiophene compounds to the corresponding *n*-butyl compounds with retention of configuration. This method provides a way for the conversion of optically active 2-thenylphenylacetic acid (V) to the corresponding α -phenyloenantic acid (IV). Since (+)-(V) gives (+)-(IV), it follows that the acids (IV) and (V) with the same direction of rotation have also the same configuration.

In an earlier paper (6) the resolution of the acids (II), (III), and (IV) has been described. Since none of the optically active acids could be obtained in the crystalline state, the amides and anilides were synthesized, rendering it possible to determine the steric relations by means of the "optical displacement rule". Thermal analysis of the nine possible two-component systems of the amides

showed that, in addition, these fulfilled the conditions for quasi-racemate formation and the configurations of the acids and of the amides could then be determined in two independent ways.

The optical displacement rule as formulated by Freudenberg (7) is an expression for the fact that compounds with the same configuration, which are closely related chemically, by conversion to derivatives of the same kind undergo a shift of the optical activity in the same direction. Undoubtedly, the acids (II), (III), and (IV) fulfil the condition of chemical proximity, and so the antipodes of these, for which the optical activities of the acids, amides and anilides can be arranged in an increasing (or decreasing) series, have the same configuration. From Table 1, in which values of $[M]_D^{25}$ in ethanol (6) are given, it is apparent that this is the case for the antipodes with the same direction of rotation.

Table 1.

	Amide	Acid	Anilide
(-)-(II) . . .	- 88.5°	- 114.0°	- 237.0°
(+)-(III) . . .	+ 80.1°	+ 120.0°	+ 221.8°
(+)-(IV) . . .	+ 95.6°	+ 124.1°	+ 228.7°

The determination of configurations by means of the quasi-racemate method has been elucidated in numerous papers from this institute. The method depends on the fact that, in certain instances, one component in a true racemate may be replaced by a chemically related substance with the same configuration (8). The resulting formation of a molecular compound (quasi-racemate) may be proved in different ways, most commonly by thermal analysis of the two-component systems. The success of the method is dependent on several factors, especially on the ability of the antipodes to form true racemates and on the structural differences between the compounds in question.

The antipodes of α -phenylvaleramide clearly have only a slight tendency for racemate formation. The system is somewhat complicated by the fact that the optically inactive amide is dimorphic, the low-melting form probably being an eutectic mixture of the antipodes (Fig. 1). The antipodes of α -phenylcaproic and α -phenyloenantic amides have a fairly good ability for racemate formation and the diagrams (Figs. 2 and 3) are simple.

In the diagrams, for which one component is an antipode of (II)-amide and the second an antipode of (III)- or (IV)-amide, complications arise from polymorphism. In Fig. 4 is shown the diagram of the amides of (II) and (III) with the same direction of rotation. The results shown strongly indicate the formation of a dimorphic molecular compound, composed of three parts of (II)-amide and one part of (III)-amide. Because of its composition, this compound is without interest relative to the question of configuration. In Fig. 5, however, the lower curve indicates a molecular compound, which, because of its composition and of the lack of a corresponding compound in Fig. 4, must be considered as a quasi-racemate. The upper curve represents a compound of more

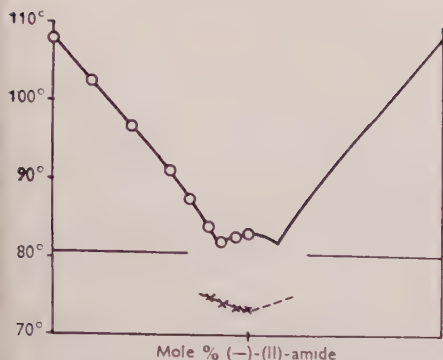


Fig. 1. (+)- and (-)-(II)-amide.

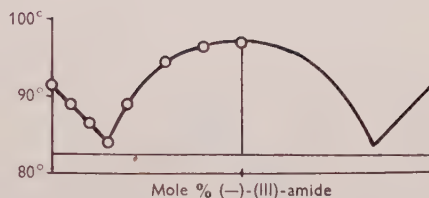


Fig. 2. (+)-(III)- and (-)-(III)-amide.

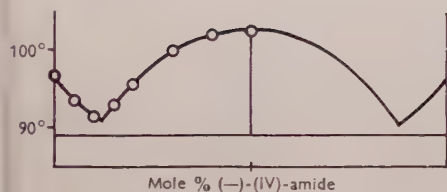


Fig. 3. (+)-(IV)- and (-)-(IV)-amide.

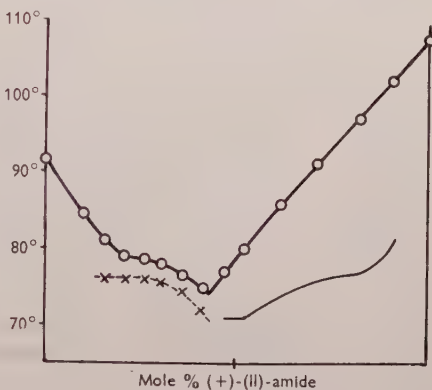


Fig. 4. (+)-(II)- and (+)-(III)-amide.

complicated structure. It seems evident, however, that the amides of (II) and (III) with the same direction of rotation have the same configuration.

In Figs. 6 and 7 are shown the two possible diagrams of the optically active amides of (II) and (IV). Fig. 6 indicates a simple eutectic system and in Fig. 7 is shown a dimorphic molecular compound, which must be considered as a quasi-racemate. Thus, the amides of the acids (II) and (IV) with the same direction of rotation have the same configuration.

The conclusion drawn from the facts shown in Figs. 4, 5, 6, and 7 must be that the amides of the acids (III) and (IV) with the same direction of rotation have the same configuration. A proof of the correctness of this is provided in the diagrams shown in Figs. 8 and 9. The antipodes with the same direction of rotation show complete mutual solubility, and the antipodes of opposite direction of rotation form a molecular compound with the composition 1:1. Therefore, the more rigid requirements, originally demanded of a quasi-racemate, are fulfilled.

The thermal analysis thus inevitably shows that the amides of the acids (II), (III) and (IV) with the same direction of rotation have all the same configuration. This is in perfect agreement with the results obtained with the optical displacement rule.

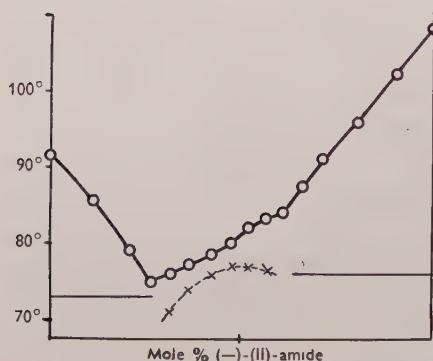


Fig. 5. $(-)$ -(II)- and $(+)$ -(III)-amide.

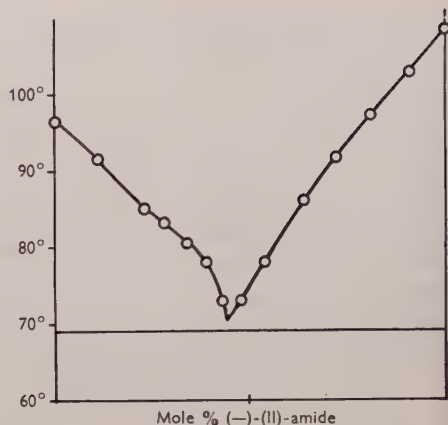


Fig. 6. $(-)$ -(II)- and $(-)$ -(IV)-amide.

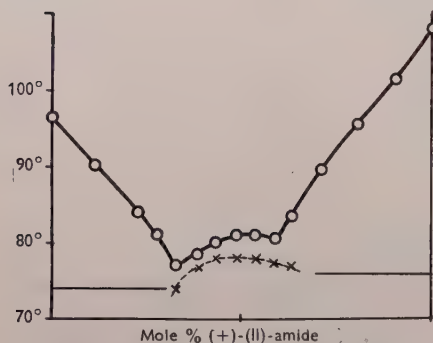


Fig. 7. $(-)$ -(II)- and $(+)$ -(IV)-amide.

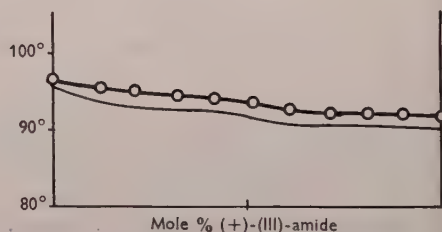


Fig. 8. $(+)$ -(III)- and $(+)$ -(IV)-amide.

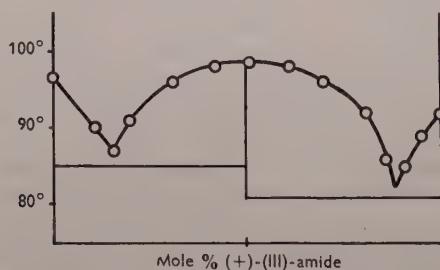


Fig. 9. $(+)$ -(III)- and $(-)$ -(IV)-amide.

It is sometimes possible to obtain information of the steric relation between two structurally allied compounds by measuring the optical activities in different solvents. If the optical activities, when progressing down the series of solvents, change in the same direction, the two compounds have the same configuration. In Table 2 are shown the molecular rotations of the acids (I)

Table 2.

Solvent	$[M]_D^{25}$				
	(+)-(I) (9)	(-)-(II) (6)	(-)-(III) (6)	(-)-(IV) (6)	(-)-(V) (1)
Acetone	+ 181°	- 157°	- 158°	- 160°	- 297°
Benzene	+ 182°	- 136°	- 144°	- 148°	- 286°
Acetic acid	+ 161°	- 128°	- 138°	- 134°	- 278°
Ether	—	- 118°	- 125°	- 124°	—
Ethanol	+ 159°	- 114°	- 119°	- 122°	- 265°
Water (ion)	+ 40°	- 5°	- 13°	- 19°	- 211°

Table 3.

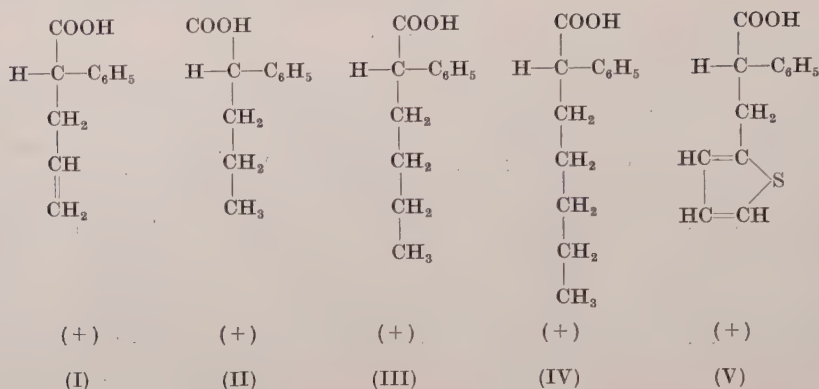
Solvent	$[M]_D^{25}$ of amide		
	(-)-(II) (6)	(+)-(III) (6)	(+)-(IV) (6)
Acetic acid . . .	- 49°	+ 56°	+ 80°
Ethanol	- 89°	+ 80°	+ 96°
Acetone	- 88°	+ 86°	+ 97°
Ether	- 163°	+ 157°	+ 190°
Benzene	- 172°	+ 161°	+ 191°

Table 4.

Solvent	$[M]_D^{25}$ of anilide		
	(-)-(II) (6)	(+)-(III) (6)	(+)-(IV) (6)
Acetone	- 184°	+ 198°	+ 192°
Acetic acid . . .	- 212°	+ 210°	+ 221°
Ethanol	- 237°	+ 222°	+ 229°
Benzene	- 338°	+ 330°	+ 348°
Ether	- 363°	+ 356°	+ 361°

through (V) in different solvents and in Tables 3 and 4 the corresponding values for the amides and anilides of the acids (II) through (IV). The results are in accordance with the results obtained with the other methods.

The summarized facts collected in this paper establish with confidence the steric relation between the acids (I) through (V), and since (+)-(I) is known to have the same configuration as D-glyceraldehyde (4), the following steric series is obtained



Experimental

Conversion of thenylphenylacetic acids to α -phenyloenantic acids

Racemic α -phenyloenantic acid: 0.5 g (0.005 moles) of racemic thenylphenylacetic acid (V) were dissolved in 5 ml of 5*N* sodium hydroxide and 100 ml of water. About 15 g of a suspension of Raney nickel in ethanol (10) were added and the mixture stirred with reflux for one hour. The Raney nickel was filtered off and the filtrate concentrated by evaporation. The residue was acidified and extracted repeatedly with ether. The ether layer was dried and the ether removed. The residue was dissolved in ethanol, neutralized with 5*N* sodium hydroxide, and treated with 1 g of *S*-benzylthiuronium chloride in ethanol. The mixture was boiled for two minutes and chilled. The sodium chloride was filtered off, the filtrate diluted with water, and allowed to crystallize in a refrigerator. The *S*-benzylthiuronium salt of racemic α -phenyloenantic acid was obtained as colourless leaves, melting at 113.5–115°. No melting-point depression was obtained when the product was mixed with an authentic sample (6).

(+)- α -Phenyloenantic acid: 0.36 g of (+)-2-thenylphenylacetic acid was dissolved in 50 ml of ethanol and the optical activity measured.

$$2 \alpha_D^{25} = +3.29^\circ, [\alpha]_D^{25} = +115^\circ (\text{lit.} +114^\circ (1)).$$

About 18 g of Raney nickel suspension was mixed with 5 ml of 5*N* sodium hydroxide and 75 ml of water and the alcoholic solution of the acid added. The mixture was refluxed with stirring for two hours. The resulting acid was isolated as *S*-benzylthiuronium salt, as described for the optically inactive acid. M.p. 116–117°. Mixed with an authentic sample (6) it showed no melting-point depression.

The benzylthiuronium salt was decomposed with dilute sulfuric acid, and the liberated acid was extracted with ether. The ether solution was washed with three portions of water and the solvent removed. 123.4 mg of the residual acid was dissolved in ethanol, made up to 10.00 ml, and the optical activity measured.

$$2 \alpha_D^{25} = +1.46^\circ, [\alpha]_D^{25} = +59.3^\circ (\text{lit.} +60.2^\circ (6)).$$

Hydrogenation of (+)-allylphenylacetic acid

Optically active allylphenylacetic acid was prepared at the laboratory by Mr. Lars Westman.

(+)- α -Phenylvaleric acid: 0.8825 g of (+)-allylphenylacetic acid was dissolved in 50.0 ml of ethanol and the optical activity measured.

$$2 \alpha_D^{22} = +3.30^\circ, [\alpha]_D^{22} = +93.5^\circ.$$

The ethanolic solution was hydrogenated in an atmosphere of hydrogen and with platinized carbon as catalyst. The catalyst was filtered off and washed with ethanol. The filtrate was concentrated to about half of its volume, neutralized with 5*N* sodium hydroxide, and treated with a solution of 1.0 g of *S*-benzylthiuronium chloride in ethanol. The mixture was refluxed for two minutes, chilled, and the sodium chloride filtered off. The filtrate was diluted with water and allowed to crystallize in a refrigerator. 1.1 g of the benzylthiuronium salt of (+)- α -phenylvaleric acid was obtained as colourless leaves, melting at 148–149° after recrystallization from dilute ethanol. A mixture of the product and an authentic sample (6) showed no melting-point depression.

The benzylthiuronium salt was decomposed, the acid isolated as described for (+)- α -phenyloenantiic acid, and the optical activity measured in ethanol.

$$112.3 \text{ mg of acid in } 10.00 \text{ ml. } 2 \alpha_D^{25} = +1.42^\circ, [\alpha]_D^{25} = +63.2^\circ (\text{lit.} +63.4^\circ (6)).$$

Melting-point diagrams

The optically active acids (II), (III), and (IV) give six optically active amides, which in turn give rise to nine different two-component systems. The results are summarized in Figs. 1 through 9 and in Tables 5 through 13.

Melting-point preparations: Weighed amounts of the components (total weight 10 mg) were dissolved in a few drops of acetone ("AnalaR" standards for laboratory chemicals). The solvent was removed by evaporation at room temperature and the residue carefully powdered and dried in a desiccator. The melting points were determined in a Kofler hot-stage microscope (11).

Table 5. (+)- and (-)-(II)-amide.

% (+)- amide	Melting point, °C	
0	106–108	
10	80–102.5	
20	80– 96.5	
30	80– 91	
35	80– 87.5	
40	–75	–83
43	–74	–82
47	–73.5	–82.5
50	–73	–83

Table 6. (+)- and (-)-(III)-amide.

% (-)- amide	Melting point, °C
0	90 –91.5
5	82.5–89
10	82.5–86.5
15	82.5–84
20	82.5–89
30	82.5–94.5
40	82.5–96.5
50	95 –97

Table 7. (+)- and (-)-(IV)-amide.

% (-)- amide	Melting point, °C
0	95.5- 96.5
5	90 - 93.5
10	89 - 91.5
15	89 - 93
20	89 - 95.5
30	89 -100
40	89 -102
50	100 -102

Table 8. (+)-(II)- and (+)-(III)-amide.

Mole-% (+)-(II)- amide	Melting point, °C
0	90 - 91.5
10	76.5- 84.5
16	-76 -81
21	-76 -79
26	-76 -78.5
31	-75.5 -78
36	-74.5 -76.5
41	-72 -75
47	71 - 77
52	71 - 80
62	74 - 86
71	76 - 91.5
82	77 - 97
91	80 -102.5
100	106 -108

Table 9. (-)-(II)- and (+)-(III)-amide.

Mole-% (-)-(II)- amide	Melting point, °C
0	90- 91.5
12	73- 85.5
21	73- 79
26	70- 75
32	-71 -76
37	-74 -77.5
42	-76 -79
48	-76.5 -80
52	-77 -82
56	-76 -83
61	74- 83.5
66	74- 95.5
72	74- 91
80	74- 95.5
91	76-101.5
100	106-108

Table 10. (-)-(II) and (-)-(IV)-amide.

Mole-% (-)-(II)- amide	Melting point, °C
0	95.5- 96.5
11	69 - 91.5
23	69 - 84
28	69 - 83
34	69 - 80.5
39	69 - 78
43	69 - 73
48	69 - 73
54	69 - 78
64	69 - 86
72	70 - 91.5
81	69 - 97
91	70 -102.5
100	106 -108

Summary

The configuration of α -thenylphenylacetic acid has been related to D-glyceraldehyde as follows:

1. (+)-2-Thenylphenylacetic acid (V) was converted to (+)- α -phenyloenantioc acid with Raney nickel.

2. The optically active α -phenyloenantioc (IV), α -phenylcaproic (III) and α -

Table 11. (–)-(II)- and (+)-(IV)-amide.

Mole-% (–)-(II)- amide	Melting point, °C	
0	95.5–96.5	
11	74 – 90	
23	74 – 84	
28	74 – 81	
33	–74	–77
38	–77	–78.5
44	–78	–80
49	–78	–81
54	–78	–81
59	–77.5	–80.5
64	–77	–83.5
73	76 – 90	
82	– 96	
90	76 –101.5	
100	100 –102	

Table 12. (+)-(III)- and (+)-(IV)-amide.

Mole-% (+)-(II)- amide	Melting point, °C
0	95.5–96.5
12	93.5–95.5
21	93 –95
32	92.5–94.5
41	92 –94
52	91.5–93.5
61	91 –92.5
71	90.5–92
81	90.5–92
91	90 –92
100	90 –91.5

Table 13. (+)-(III)- and (–)-(IV)-amide.

Mole-% (+)-(III)- amide	Melting point, °C
0	95.5–96.5
11	85 –90
16	85 –87
20	85 –91
31	85 –96
42	85 –98
51	93 –98.5
61	81 –98
70	81 –96
81	81 –92
86	81 –86
91	81 –85
95	81 –89
100	90 –91.5

phenylvaleric acids with the same direction of rotation were shown to have the same configuration by means of the optical displacement rule, applied to the acids, amides and anilides. The same steric relation was found between the amides of the acids by means of the quasi-racemate method.

3. (+)-Allylphenylacetic acid (I), sterically related to D-glyceraldehyde, was catalytically hydrogenated to (+)- α -phenylvaleric acid.

4. A comparison of the optical activities of the acids (I) through (V) and of the anilides and amides of the acids (II), (III), and (IV), when measured in different solvents, verifies the results obtained in points 1, 2, and 3.

The author is indebted to Prof. Arne Fredga for his stimulating interest in the present work and for many valuable discussions, and to Mr. Lars Westman, who prepared the sample of the (+)-allylphenylacetic acid used.

Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, and Pharmacia Research Laboratory, Uppsala, December 1955.

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Properties of ethyl hydroxyethyl cellulose molecules in solution

By R. ST. JOHN MANLEY

With 28 figures in the text

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Preface

The work to be described in the following pages has been carried out at the Institute of Physical Chemistry of the University of Uppsala, Sweden, under tenure of a post doctoral research fellowship from the National Research Council of Canada. I am indebted to them for providing me with this opportunity of broadening my research experience.

I wish to express my deep gratitude to the director of the Institute, Professor Stig Claesson, for his great interest in the work, for his kindly advice and encouragement and for placing the excellent facilities of the Institute at my disposal.

My sincere appreciation is also due to Dr. Ingvar Jullander for his unfailing interest and cooperation and for many valuable discussions.

The routine calculations in connection with the sedimentation and diffusion experiments have been performed by the staff of the calculating department at the

Institute. I wish herewith to express my warmest thanks to these and other members, past and present, of the Institute who on numerous occasions have given me the benefit of their judgement or of their aid and who have constantly provided the moral support of friendship and interest in this work.

Finally I wish to acknowledge my indebtedness to Mo och Domsjö AB., Sweden for the many ways in which they have aided this investigation.

Introduction

Cellulose is one of the most abundant of all naturally occurring organic substances and has very great importance both technically and economically. From a purely scientific viewpoint the study of cellulose provides many research problems; during recent decades cellulose and its derivatives have been the subjects of intense investigation and many important questions have been answered although great difficulties, both of an experimental and theoretical nature have been encountered. An extensive survey of our present knowledge in this field has been given by Ott (1). In the following, a short orientation is given as an introduction to the present work.

The chemical structure of cellulose is now well established. The cellulose molecule can be considered as resulting from a 1-4 polycondensation of beta-glucose to form a chain molecule composed of a large number of six membered rings, normally called glucose residues. The number of glucose residues in a cellulose molecule may be of the order of several thousand. Within a cellulose fiber the chain molecules are bound laterally to another by hydrogen bonds in such a way as to form crystallites (small crystalline areas) separated by amorphous regions. The reactivity of the cellulose in the amorphous regions is greater than in the crystallites. Although the cellulose molecule has been widely investigated no definite conclusion can be drawn as to its size. Existing methods for the determination of the degree of polymerisation have given results varying from about 200 for viscose silk to 5000 for native cotton cellulose. All the known methods of dissolving cellulose are assumed to cause depolymerisation to a greater or lesser degree; cellulose is known to be polymolecular in solution and possibly is so also in its native state.

In solution, molecules of cellulose and its derivatives possess an unbranched thread-like form. Depending upon the solvent, the temperature and the degree of polymerization a given cellulose molecule may assume different configurations ranging from that of a completely randomly coiled chain to that of an almost linear rod. However, in recent years it has been shown that, compared with most other polymers, the molecules of cellulose and its derivatives tend to be relatively rigid in solution.

From an organic chemical point of view cellulose can be regarded as an alcohol; it undergoes some of the reactions characteristic of this group of substances—in particular ether and ester formation. The esters are by far the most important and well known of the cellulose derivatives. The best known representatives of this class are cellulose nitrate and cellulose acetate, both of which were discovered already around the middle of the nineteenth century.

The ethers of cellulose are considerably more recent. Broadly speaking, two types may be distinguished: cellulose ethers soluble in organic media and cellulose ethers soluble in water and alkali. The present study is concerned with a cellulose ether and we therefore restrict our attention to this class.

The etherification process generally consists of the preparation of alkali cellulose by interaction of cellulose with a base and solvating agent and the reaction of the alkali cellulose with an etherifying agent (e.g. an alkyl halide). Byproducts of the reaction are removed by washing with methanol-water or ethanol-water solutions or in some cases with boiling water. After drying, the product is obtained in a flaky or powdery form of white or yellow-white colour.

In all cases there is no certainty as to the position of the substituents in the anhydroglucose units. This problem has been discussed by Timell (2). Equally scant is our knowledge of the distribution of substituents along the chain.

The properties of cellulose ethers vary with the degree of substitution, with the degree of polymerization and the nature of the substituent. The solubility of cellulose ethers is largely controlled by the degree of substitution. Cellulose itself is not water soluble, despite the fact that it contains a larger number of hydroxyl groups. This is due to the fact that the molecules are strongly bound to one another by hydrogen bonds. In alkali, cellulose swells but does not dissolve. The partial replacement of the hydroxyl groups by more bulky substituents serves to break up the superstructure, thereby weakening the hydrogen bonds so that solubility in water becomes possible. With increasing degree of substitution a given ether will be soluble first in alkali, then in water and finally in organic solvents.

The solubility of cellulose ethers is also influenced by the distribution of the substituents. If the groups are uniformly distributed along the chain, better solubility will be observed than if complete substitution occurs in some sections of the chain alternating with sections of no substitution. In regions where there is no substitution, packing similar to cellulose itself will exist, provided the sections are long enough, with the result that such sections will resist solution and hence interfere with complete solubility.

The degree of polymerization of cellulose ethers can also be varied within wide limits. It depends partly on the type of cellulose used as the starting material and partly on the temperature of reaction. The nature of the substituent also plays an important role in determining the properties of the ether. One may distinguish between ionic and non-ionic ethers. The best known representative of the former group is carboxymethyl cellulose. Members of the latter class contain methyl, ethyl or hydroxyethyl groups either singly or in various combinations.

A characteristic of all non-ionic water soluble cellulose derivatives is that they cause a very strong decrease in the surface tension of water and they are therefore used as emulsifiers. The marked tendency of the solutions to foam makes experiments somewhat more difficult. Finally it may be mentioned that water soluble cellulose ethers which contain ethyl or methyl groups are soluble only in cold water. On warming, flocculation or gelation occurs but on cooling, the ether again becomes soluble. A description of the preparation and properties of water soluble cellulose ethers has been given by Jullander (3).

In comparison with cellulose esters very few studies have been made of the solution properties of cellulose ethers. The molecular weight of carefully fractionated samples of water-soluble methyl cellulose was determined by Signer and coworkers (4, 5) and Polson (6) from sedimentation equilibrium, viscosity and diffusion. Osmotic pressure and viscosity measurements on similar methyl celluloses have been made by Staudinger and coworkers (7). More recently Jullander (8) has described some of the properties of ethyl hydroxyethyl cellulose.

The effect of salt on solutions of these ethers has been studied by a number of

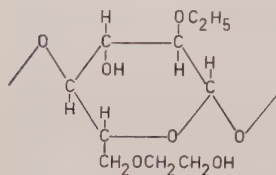


Fig. 1. Schematic structural formula of the repeating unit in ethyl hydroxyethyl cellulose.

authors (9-13). The addition of salt causes dehydration of the cellulose chains and at sufficiently high salt concentrations flocculation or gelation occurs. The ability of various salts to cause flocculation generally follows the Hofmeister series.

As has been emphasised by Jullander (8), water soluble cellulose derivatives never dissolve completely. In addition to the molecularly dispersed cellulose chains a solution contains molecular aggregates of submicroscopic size, gel particles and undissolved fibres. This fact has seldom been taken into consideration in physico-chemical investigations of the properties of water soluble cellulose derivatives and may certainly be expected to lead to difficulties. The same phenomena is observed in solutions of other polymers, for example rubber both of natural and synthetic origin and viscose. In the former case it is due to cross-linking and in the latter to the different reactivity of the crystalline and amorphous regions in the cellulose.

This paper gives an account of an investigation of a non-ionic water soluble cellulose derivative namely ethyl hydroxyethyl cellulose. The schematic structural formula is shown in Fig. 1. It is a mixed ether prepared by the reaction of alkali cellulose with a mixture of ethylene oxide and ethyl chloride in an autoclave. Details of the preparation have been given by Sönnerskog (12). It is manufactured commercially by Mo och Domsjö AB, Sweden, and is marketed under the trade name of *Modocoll*. Its properties, which are similar to those of other cellulose ethers, have been described by Jullander (8, cf. also Sönnerskog, 12). One property deserves emphasis at this point. When an aqueous solution of ethyl hydroxyethyl cellulose prepared at room temperature is cooled to about 5°C, the viscosity increases irreversibly and the solution becomes considerably clearer. This is probably partly due to increased molecular solvation at low temperatures and partly also to the low temperature dissolution of fibers which are inhomogeneously etherified or have a very resistant structure (8). On heating, the viscosity decreases and gelation begins at a temperature of about 50°C.

Ethyl hydroxyethyl cellulose is a relatively new polymer whose physico-chemical properties have not been previously investigated. In some respects the substituent groups have quite different characteristics, for example, the ethyl group is hydrophobic, while the hydroxyethyl group is hydrophilic. It was of interest to see whether this would confer any unique properties upon the behavior of the molecules in solution. Furthermore, because of the fact that the polymer is water soluble and non-ionic its thorough study might be expected to provide background information for the theoretical treatment of the more complicated polyelectrolyte systems based upon the cellulose skeleton.

In order to obtain as complete a characterization as possible, a wide variety of methods has been used. The goal was not to make precision measurements of molecular parameters, but rather to try to get a broad overall view of the molecular behavior in solution. Studies have been made of the viscosity, sedimentation, diffusion, osmotic pressure and light scattering of various samples prepared by

fractionation. In this way a series of molecular weight averages is obtained and the many methods used therefore serve not only as a mutual check on the reliability of the results but also as a means of estimating the breadth of the molecular distributions in the samples. As will be seen later this latter point is of considerable importance in evaluating the data. Furthermore, the relation between the various molecular constants, intrinsic viscosity, sedimentation and diffusion coefficients and the molecular weights give information about the shape of the molecules.

The aim of the work has also been to determine the manner in which the data obtained fit into the frame of current theories on the behavior of flexible linear macromolecules in dilute solution and to interrelate some of the results in terms of fundamental molecular parameters.

The work is presented in two parts, the first of which deals with various aspects of the experimental work. Whenever possible, comparisons are drawn to corresponding data available in the literature. The second part is devoted to a general discussion of the experimental results and their evaluation in the light of current theory.

PART I

Experimental procedures and results

(1) Fractionation of ethyl hydroxyethyl cellulose

The material used was a commercial sample (Modocoll E 600)¹ and was used without further purification. Analytical data are given in Table 1.

As is well known, the fractionation of high polymers largely depends on the very small decrease in solubility with increase in molecular weight in a given polymer

Table 1. Fractionation of ethyl hydroxyethyl cellulose.

Fraction No.	wt. of fraction g	Extractant acetone-H ₂ O wt. %	$[\eta]_{25}$ dl/g	Ethoxyl %	Ethoxyl + hydroxyethyl %	NaCl %
Original	—	—	6.2	18.0	33.3	0.9
1	18.6	80	1.30	21.8	25.6	3.2
2	16.9	65	2.60	19.6	31.5	0.1
3	24.6	50	3.49	18.5	31.1	0.1
4	26.4	45	4.20	18.3	31.5	0.1
5	19.5	39	4.70	17.9	29.1	0.3
6	22.0	34	5.70	17.8	28.4	0.2
7	24.4	25	6.75	17.5	30.0	—
8 A	13.9	20	7.90	17.1	29.9	—
8 B	14.3	20	7.00	17.1	32.5	—
9	31.3	15	8.70	17.4	29.5	—
10	30.7	10	8.90	17.6	29.9	0.1
11	15.7	5	8.90	17.6	30.0	0.3
12	20.1	0	9.10	18.0	29.6	0.1

¹ Manufactured by Mo och Domsjö AB, Sweden.

series. Two methods have generally been used, fractional precipitation from a dilute solution and fractional extraction.

In the case of cellulose derivatives the solubility depends not only on the degree of polymerization but also on the chemical composition. However, a proper choice of solvent-non-solvent systems may result in fractionation being effected mainly according to one of these factors.

In the case of the material under investigation the problem was complicated by the fact it is only soluble in water. This had the effect of severely limiting the choice of non-solvents for fractional precipitation. Methanol, ethanol, propanol, dioxane, acetone and solutions of sodium chloride and sodium sulphate proved to be inefficient and great difficulties were experienced in isolating the precipitated phase which remained dispersed even after prolonged centrifugation at about 2000 r.p.m.

These difficulties were surmounted by the use of a fractional solution method. The solid material retained in finely divided form was extracted isothermally (20°C) with acetone-water mixtures of increasing solvent power, the polymers of lowest molecular weight being extracted first from the sample. So as to ensure that a sufficient quantity of each fraction would be available for the large number of experiments envisaged, 500 g of starting material was used. In order to get maximum efficiency of separation large volumes of extractant (15–20 litres) were used and the extraction was allowed to continue until equilibrium was established. This condition was considered to exist when two or three successive gravimetric analyses (evaporation to constant weight of an aliquot) taken over intervals of twenty-four hours showed a constant amount of material in solution. The establishment of this equilibrium took rather a long time varying from three days for the low molecular weight fractions to approximately two weeks for fractions of higher degree of polymerization.

After the attainment of equilibrium the solution was separated by centrifugation and the residue treated with an extractant containing less acetone. At the end of the fractionation there remained an insoluble residue probably consisting of partially etherified fibers.

With the exception of the first fraction, which was concentrated under vacuum at 30°C and then freeze-dried, the polymer in the acetone-water solution was coagulated by the addition of excess acetone (approx. 4 pts. acetone to 1 pt. soln.). The polymer settled spontaneously and the supernatant liquid was decanted. The material was then dried at 35°C under vacuum after exhaustive washing with ethanol followed by benzene. Material dried under similar conditions directly from water produced a hard horny mass which was difficult if not impossible to redissolve.

Twelve fractions were thus obtained, each weighing about 25 g, and were designated M1–M12, 12 being that of highest degree of polymerization. The average degree of substitution (see Fig. 1) was 0.56 with respect to ethoxyl (OC_2H_5) and 0.84 with respect to ethylene oxide (OC_2H_4) and varied slightly from fraction to fraction. The corresponding average base molar weight is 210.

An interesting fact which perhaps deserves mention is that as the fractionation proceeded, increasing difficulty was experienced in effecting quantitative coagulation of the polymer in solution. Generally there was about 90 per cent recovery, the remainder of the material being dispersed in the liquid phase regardless of how great an excess of acetone was added. Thus in fraction 8 only 50 per cent of the material was recovered by coagulation. However, it was discovered thereafter that the remainder of the material in the supernatant phase could be quantitatively precipitated by the addition of a very small quantity of sodium chloride solution

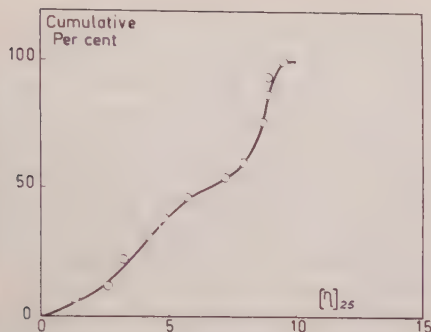


Fig. 2.

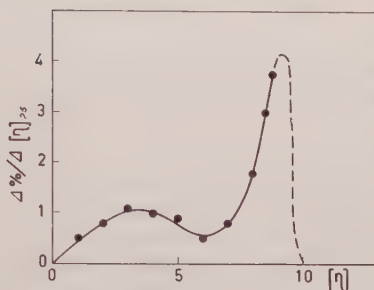


Fig. 3.

Fig. 2. Integral distribution curve for the unfractionated sample of ethyl hydroxyethyl cellulose. The curve is constructed from the weights and intrinsic viscosities of the fractions.

Fig. 3. Differential distribution curve for the initial sample of ethyl hydroxyethyl cellulose as obtained by graphical differentiation of the integral curve (Fig. 2).

(1 c.c. 0.5 per cent NaCl solution per 20 litres of polymer solution). The effect seems to be connected with the fact that with each successive extraction more and more of the salt present in the original material was washed out, thus making it possible for a part of the polymer to become colloiddally dispersed. The degree of polymerization and degree of substitution of the two parts of the fraction were quite different.

The ethoxyl content of each fraction was determined after Lemieux and Purves (14). The material is oxidized with chromic acid solution, the ethoxyl groups being transformed into acetic acid which is distilled off and titrated. The sum of the ethoxyl and hydroxyethyl groups was determined by the modified alkoxy group method described by Morgan (15). The hydroxyethyl content was obtained as the difference between the ethoxyl content and the total substitution. The salt content was determined by titration against silver nitrate.¹ The intrinsic viscosity of each fraction was measured with an Ubbelohde viscometer. Details of these measurements will be given later.

The results of the fractionation are shown in Table 1. It would appear that the method of fractionation lacks sensitivity in the high molecular weight range there being little or no separation between fractions 10–12. The material of low degree of polymerization is most highly substituted but within the limits of experimental error the degree of substitution is constant in the medium and high molecular weight ranges.

Molecular distribution curves for the starting material, constructed on the basis of the fractionation, are shown in Figs. 2 and 3. It is evident that the sample was extremely polydisperse.

(2) Preparation of solutions

Fractions M3, M5, M7 and M12 were selected for measurements and were used without further purification.

All measurements were made from standard solutions which were prepared as follows: To a weighed quantity of the polymer about 25 per cent of the volume of

¹ The author is indebted to Mr. O. Lagerström at the Laboratories of Mo och Domsjö AB, Sweden, for carrying out these analyses.

water needed to produce the required concentration was added as hot water (80–100°C) and the container was maintained at this temperature for about thirty minutes. Cold water (< 10°C) was then added to produce the required concentration. The addition of hot water causes swelling of the fibers thereby hastening the solution process upon the addition of cold water. This treatment had the further advantage that it helped to minimise the formation of gel around the solid particles, a phenomena frequently encountered in the dissolution of high polymers.

The dispersion thus formed was vigorously shaken for about 48 hours in a cold room at 4°C. As the viscosity of a solution depends on the lowest temperature to which it is subjected (8), all solutions were then frozen and later thawed out.

Preliminary experiments indicated that small amounts of salt could have an effect upon the viscosity behavior, a 10 per cent decrease in $[\eta]$ being obtained in the range $0\text{--}30 \times 10^{-4}$ molar NaCl. This effect seems to be similar to that observed by Pals and Hermans (16) for aqueous solutions of polyvinylalcohol and is probably the result of ion adsorption. Accordingly all solutions were rendered salt free by passage through an ion-exchange column containing a mixed bed (Dowex 50—acid form and Amberlite IRA 400—base form). The solutions were sufficiently dilute that there were no difficulties due to clogging of the column. By measuring the viscosity of a solution, freed from salt by electrodialysis, before and after passage through a similar column it was established that there was no selective adsorption of polymer species on the resin.

The salt free solutions were cleaned by centrifugation in the large rotor of a Spinco preparative ultracentrifuge (30,000 r.p.m. for 2 hours). In all cases a small gel-like deposit was obtained. The solutions were then stored in a refrigerator at 4°C. Fresh solutions were prepared about once per month.

In all experiments concentrations were determined by evaporating a portion of the solution to constant weight at 105°C. A conversion to units of grams per 100 c.c. was made from a knowledge of the density of the solution. This method is known to be inaccurate in certain cases (17), since the film of polymer that is formed on the walls of the containing vessel may still retain an appreciable percentage of volatile material. In the present case, the fact that the heating of the solution causes precipitation means that there is no “glass” formation and the solvent is easily and completely removed by the evaporation. Constant weight was attained in 6–8 hours.

(3) Viscosity

All viscosity measurements were made with an Ubbelohde type capillary viscometer. The non-Newtonian behavior of the solutions was taken into account by constructing the viscometer with four bulbs at different heights to allow measurements at four gradients. A large reservoir (120 c.c.) was provided so that dilutions could be carried out in the instrument. In order to avoid difficulties caused by dust a capillary of relatively large radius was used. The capillary was coiled in the form of a wide smooth loop in a vertical plane. It was made of precision Hysel tubing the radius of which was determined by measuring the weight of mercury in a given length. The design of the instrument is similar to that used by several authors (cf. e.g. ref. 18). The dimensions of the instrument are given in Table 2.

The mean hydrostatic head, h_m , was calculated from the formula (19)

$$h_m = \frac{h_1 - h_2}{\ln h_1/h_2}$$

Table 2. Viscometer dimensions and constants.

Efflux bulb No.	1	2	3	4
Efflux volume (c.c.)	2.50	2.01	1.21	0.60
Mean hydrostatic head (cm) . . .	28.6	23.7	14.4	6.72
Flow time for water at 25°C (sec)	152.1	146.2	140.3	155.5
Surface tension constant				
$C \times 10^4$ c.g.s. units	1.3	0.2	-1.0	-3.8
$\alpha \times 10^3$	5.912	6.143	6.392	5.741
β	0.9799	0.7620	0.4378	0.7702
β/α	163.7	124.0	68.5	-134.2

Capillary length: 30.0 cm.

Capillary radius: 0.50 mm.

where h_1 and h_2 are respectively the distances between the upper and lower mark on each bulb, and the lower end of the capillary.

The viscosity, η , may be expressed in the form:

$$\frac{\eta}{d} = \alpha(t - \beta/\alpha t), \quad (1)$$

the constants α and β being given by

$$\alpha = \pi r^4 h g / 8 V l$$

$$\beta = m V / 8 \pi l$$

where h is the mean hydrostatic head, g is the acceleration due to gravity, d is the liquid density, V is the volume of liquid delivered in time t , r is the radius of the capillary, l its length, and m is an end constant depending, among other things, on the shape of the ends of the capillary (usually taken as 1.1).

The constants α and β were determined by measuring the efflux time for water at 0, 15, 25 and 40°C. By plotting $\eta t/d$ (using values of η and d taken from the International Critical Tables) against t^2 a smooth straight line was obtained which, fitted by the method of least squares, gave α and β as the slope and intercept respectively. Values obtained for each bulb are given in Table 2. Wherever necessary the small kinetic energy correction (highest value about 0.8 per cent for solutions at 40°C) was applied using the relation

$$\frac{\eta_s}{\eta_0} = \frac{d_s t_s}{d_0 t_0} \left[1 + \frac{\beta}{\alpha} \left(\frac{1}{t_0^2} - \frac{1}{t_s^2} \right) \right] \quad (2)$$

where the subscripts s and 0 refer to solution and solvent respectively.

The mean velocity gradient $\bar{G}$ in the viscometer was calculated from the formula of Kroepelin (20)

$$\bar{G} = \frac{8V}{3\pi r^3 t}, \quad (3)$$

the symbols having the aforementioned significance. For the solutions used the values calculated covered a range 50–1000 sec⁻¹.

As previously mentioned the solutions under investigation are highly surface active and it was therefore considered necessary to investigate the possible effect of this on the viscosity measurements. As there are only relatively few discussions of such effects in the literature (21, 22, 23) the results are given in some detail. The method used resembles that described by Philippoff (23) and is based upon the following considerations.

In a capillary of radius r the capillary rise due to surface tension is given by the well-known formula $2\gamma = h d g r \cos \theta$, γ being the surface tension of the liquid, h the capillary rise, θ the angle of contact of the liquid with the walls of the capillary, and the other symbols have the usual significance. The surface tension causes a change in the efflux time which can be represented by the relation

$$t_{\text{obs}} = t_{\text{corr}} H_0 \left/ \left(H_0 - \int_{h=H_0}^{h=H_e} \frac{2\gamma}{d g r \cos \theta} dr \right) \right. \quad (4)$$

where the integral is taken over the limits from the initial (H_0) to the equilibrium height (H_e) and t_{obs} and t_{corr} are the observed and corrected efflux times respectively.

If the integral is incorporated in an apparatus constant, C , it may be readily shown that for dilute solutions

$$(\eta_r)_{\text{obs}} = (\eta_r)_{\text{corr}} \left[1 + C \left(\frac{\gamma_0}{d_0} - \frac{\gamma_s}{d_s} \right) \right] \quad (5)$$

where η_r is the relative viscosity and the subscripts have the above mentioned significance. γ_0 and γ_s are the surface tensions of solvent and solution respectively and d_0 and d_s the corresponding densities.

The value of C was determined by measuring the relative viscosities of a number of dilute solutions of sodium oleate and Teepol (a synthetic cationic detergent) and extrapolating the data for each of the four bulbs to infinite dilution.

If the surface tension is independent of concentration in the concentration interval investigated, it is clear that in the absence of disturbing factors the extrapolated value of η_r must be unity and the fact that it is not so may be attributed to surface tension effects. Measurements were made at 25°C. The results for Teepol are shown in Fig. 4. Thus, setting $(\eta_r)_{\text{corr}} = 1$ and taking $(\eta_r)_{\text{obs}}$ from Fig. 4 the magnitude of C may be calculated if the surface tensions of the solutions are known. These were measured by the capillary rise method. Over the concentration range investigated (0.1–0.4 per cent for sodium oleate and 0.5–2.0 per cent for Teepol) the surface tensions were essentially constant and the value in this range (26 and 32 dynes/cm for sodium oleate and Teepol respectively) was used to evaluate C . The values of C obtained with sodium oleate and Teepol agreed to within 1 per cent except for measuring bulb 2 where there was a 3 per cent difference. The mean values given in Table 2 were used for further calculations. The sign of the constant C indicates whether the efflux time is increased or decreased by the surface tension. These effects depend upon the relative size and shape of the meniscus in the measuring bulb and lower end of the capillary respectively; the efflux time may be increased or decreased depending upon this factor.

The surface tensions of the polymer solutions were also measured by the capillary rise method.

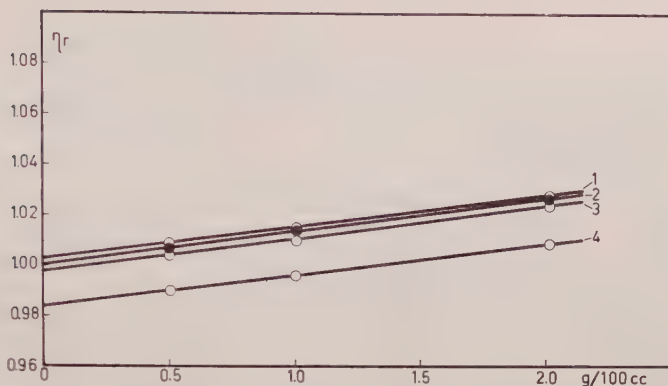


Fig. 4. Calibration of Ubbelohde viscosimeter for surface tension effects. Data for efflux bulbs no. 1, 2, 3 and 4 respectively.

The viscosities of the polymer solutions were all measured in the concentration range 0–0.3 per cent at the high end of which the surface tension showed a decrease from 71 to 56 dyne/cm. From this it was calculated that the maximum corrections would be 0.2, 0.03, 0.1 and 0.6 per cent for measuring bulbs 1–4 respectively. For bulbs 1–3 the correction lies within the experimental error and was therefore neglected, but for bulb 4 it was applied wherever necessary.

Viscosity measurements were made on a given solution by moving the viscometer between four thermostats controlled to $\pm 0.01^\circ\text{C}$ at temperatures of 0, 15, 25, and 40°C , the flow times being recorded to 0.1 sec. for each of the four bulbs. Dilutions were made by volume in the viscometer. All solutions were filtered directly into a special pipette having negligible drainage losses and thence transferred to the viscometer. The concentrations used were so chosen as to yield relative viscosities η_r in the range from 1.1 to 1.7.

For each concentration the relative viscosity of the solution in each of the four bulbs (corrected if necessary for kinetic energy and surface tension) was plotted against the rate of shear calculated from equation (3). The best straight line was drawn through the points and extrapolated to zero gradient. Some typical examples are illustrated in Fig. 5. In almost all cases the relative viscosity obtained from measurements in bulb 4 (i.e. at the lowest velocity gradients) lay below the line through the values from the other three bulbs. This is probably caused by a systematic error and accordingly this point was neglected in the extrapolation to zero gradient. In all cases the gradient dependence was very small and as expected the dependence decreased with temperature.

After correction to zero rate of shear the η_r values were used for calculating η_{sp}/c and $(\ln \eta_r)/c$ both of which were then plotted against concentration as illustrated by the examples shown in Fig. 6. The intrinsic viscosity was taken as the common ordinate intercept for the straight lines drawn through the two sets of points; their slopes being related to one another in accordance with the mutually related equations

$$\eta_{sp}/c = [\eta] + k[\eta]^2 c \quad (7)$$

$$(\ln \eta_r)/c = [\eta] - (0.50 - k)[\eta]^2 c \quad (8)$$

where k is a constant characteristic of a given solvent–polymer pair and independent of the polymer molecular weight. Values found for k are given in Table 3. In all

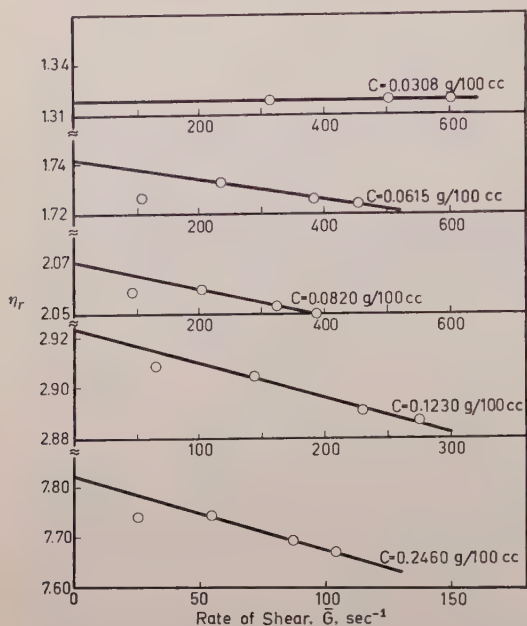


Fig. 5. Relative viscosity as a function of velocity gradient for ethyl hydroxyethyl cellulose (fraction M12) in water at 20°C. In the extrapolation to zero gradient the points at the lowest gradient are neglected (see text).

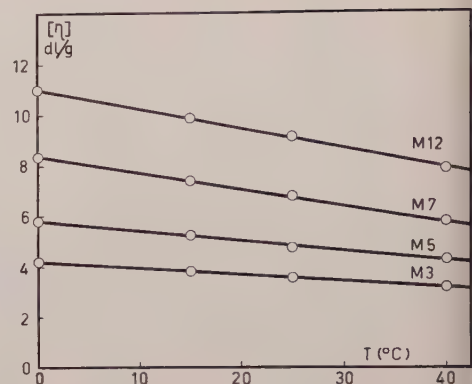


Fig. 7. Intrinsic viscosity-temperature relationships for four fractions of ethyl hydroxyethyl cellulose in water.

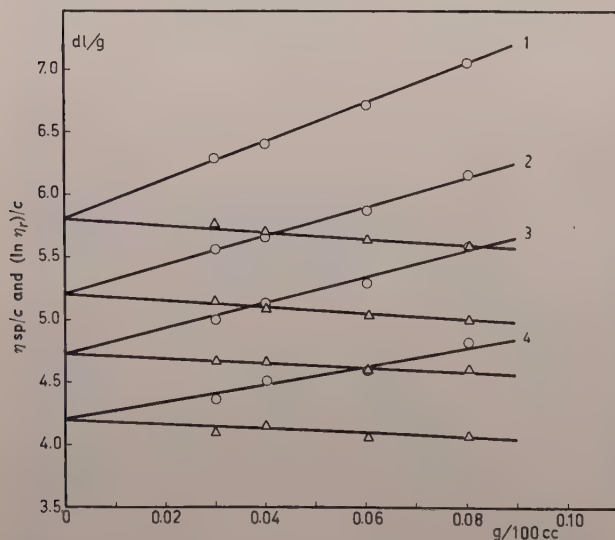


Fig. 6. The dependence of η_{sp}/c (circles) and $(\ln \eta_r)/c$ (triangles) on concentration for a fraction (M5) of ethyl hydroxyethyl cellulose in water at temperatures of (1) 0°C, (2) 15°C, (3) 25°C and (4) 40°C.

cases these plots were linear. The intrinsic viscosities are summarized in Table 3 and the complete experimental data are presented in Table 4. Plots of $[\eta]$ vs. T are shown in Fig. 7. The significance of the results will be considered later after the presentation of the molecular weight determinations.

Table 3. Intrinsic viscosity-temperature relations.

Fraction No.	Temp. °C	Incr. in $[\eta]$ on extrapolating to zero gradient %	k	$[\eta]$ dl/g	Temperature coeff. $\Delta [\eta]/\Delta T$ (dl/g deg.)
M12	0	5.8	0.50	11.00	0.080
	15	5.4	0.43	9.86	
	25	5.2	0.39	9.10	
	40	0	0.46	7.80	
M7	0	1.2	0.67	8.35	0.066
	15	0	0.64	7.38	
	25	0	0.66	6.75	
	40	0	0.75	5.70	
M5	0	0	0.44	5.80	0.040
	15	0	0.41	5.20	
	25	0	0.45	4.70	
	40	0	0.39	4.20	
M3	0	0	0.54	4.20	0.027
	15	0	0.55	3.78	
	25	0	0.51	3.49	
	40	0	0.50	3.10	

Table 4. Viscosity measurements for ethyl hydroxyethyl cellulose in water at different temperatures.

Fraction No.	c g/dl	η_{sp}/c (dl/g)			
		0°C	15°C	25°C	40°C
M3	0.1045	5.19	4.54	4.13	3.60
	0.0690	4.87	4.35	3.90	3.42
	0.0517	4.71	4.19	3.77	3.30
	0.0345	4.52	4.14	3.68	3.27
M5	0.1210	7.84	6.70	6.00	5.17
	0.0807	7.04	6.15	5.58	4.82
	0.0605	6.71	5.87	5.29	4.59
	0.0403	6.40	5.66	5.14	4.52
	0.0302	6.29	5.56	5.00	4.37
M7	0.1019	13.15	10.94	9.77	8.23
	0.0679	11.45	9.73	8.78	7.36
	0.0509	10.65	9.19	8.21	6.95
	0.0407	10.20	8.80	7.98	6.88
	0.0339	9.94	8.55	7.76	6.73
M12	0.0823	16.40	13.81	12.34	10.32
	0.0549	14.32	12.15	10.80	9.34
	0.0412	13.39	11.54	10.52	8.87
	0.0274	12.57	11.01	9.98	8.45
	0.0296	12.29	10.69	9.81	8.70
	0.0103	11.95	10.30	9.43	8.75

(4) Osmotic pressure

Osmotic pressure measurements on solutions of macromolecules are of interest primarily because they can be used for the determination of number average molecular weights, $\bar{M}_n$.

According to the classical van't Hoff law, the osmotic pressure of a dilute ideal solution is given by

$$\pi = RTc/M \quad (9)$$

where π is the osmotic pressure, c the concentration of the solution, and M the molecular weight of the solute. In the case of polymer solutions, due to intermolecular interaction, there are large deviations from ideality even at low concentrations. The reduced osmotic pressure π/c increases with concentration and extrapolation to infinite dilution is necessary. This extrapolation is often complicated by the fact that the $\pi/c = f(c)$ relation is curved. On the other hand the relation is likely to be linear at very low concentrations.

The Flory Huggins theory of polymer solutions (24, 25) has provided the basis for the interpretation of the nonideal behavior of polymer solutions. According to this theory the reduced osmotic pressure may be expressed in the form

$$\frac{\pi}{c} = \frac{RT}{\bar{M}_n} + RT d_1 (0.5 - \mu) c / M_1 d_2^2 + \cdots \quad (10)$$

where d_1 and d_2 are the densities of solvent and solute, M_1 is the molecular weight of the solvent and μ is a solvent-solute interaction constant. This equation may also be regarded as a power series expansion of π/c which can be written in the form

$$\pi/c = RT [A + Bc + \cdots] \quad (11)$$

where A and B , by analogy with the theory of real gases, are known as the first and second virial coefficients respectively. In practice, terms beyond Bc are neglected.

The second virial coefficient B is related to the solvent-solute interaction and according to Flory and Krigbaum (26) is dependent on the molecular weight of the polymer and upon molecular weight heterogeneity.

Although several theoretical equations have been proposed to account for the variation of the reduced osmotic pressure with concentration, there is at present no completely satisfactory answer to the question. A review of recent developments has been given by Wall and Hiller (27).

Experimental technique and results

In choosing an instrument for carrying out the osmotic pressure measurements two problems had to be borne in mind; namely, the high surface tensions of the solutions which would necessitate large uncertain corrections to the observed osmotic pressures, and the probability of a non-linear variation of the reduced osmotic pressure with concentration, leading to difficulties in extrapolation. Both problems were avoided by the use of the interference osmometer developed by Claesson and Jacobsson (28, 29, 30). This instrument allows measurements to be made at very low concentrations and surface tension effects are eliminated by the use of wide

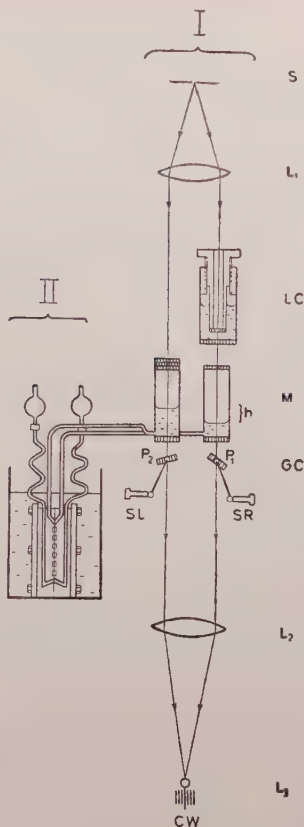


Fig. 8. Drawing showing the principal features of the interference osmometer (after Claesson, 29).

(20 mm) manometric tubes. A drawing of the apparatus is shown in Fig. 8. The osmotic cell is of the conventional Fuoss Mead type (31) in which the usual capillaries are replaced by the wide manometric tubes. A pair of light beams originating from a white light source, *S*, pass through the manometric tubes and produce a set of interference fringes in an ocular *L* 3. Changes in the difference between the liquid levels (*h*) in the manometer tubes cause a movement of the interference fringes in the ocular. This movement is followed by adjusting the glass compensators *SL* and *SR* and the liquid compensator *LC*, all of which are calibrated in terms of millimeters. The instrument is capable of an accuracy of about 10^{-3} mm of water pressure. The osmotic cell is mounted in a thermostat in which the temperature fluctuations are less than $\pm 0.005^\circ$. The membrane used was an UltrafeinfILTER from Membranfilter Gesellschaft, Göttingen, labelled 15. Before use it was conditioned in water for a period of about one week. Measurements were carried out by a dynamic method previously used by Gee (32), Montanna and Jilk (33) and Jullander (38). The rate of solvent flow through the membrane is given by the relation

$$dh/dt = K(h - \pi). \quad (12)$$

Thus if dh/dt is determined at different positions *h*, and the data plotted, a straight line is obtained from which the osmotic pressure π , that is, the value of *h* at $dh/dt = 0$, can be found.

The reference position (i.e., the cell constant K) was first determined with water in both halves of the cell. Measurements were then made on the solutions in increasing order of concentration. The techniques of filling the cell and exchanging solutions were the same as described by Jacobsson (30). The latter author had found that the membrane could be adequately conditioned by allowing about 100 c.c. of solution to flow slowly through the cell over a period of about one hour. With the present polymer system a much longer time was required. The sequence of operations used in the present study was as follows. The solution half cell was first flushed with about 100 c.c. of the solution to be measured, and after leaving this solution in contact with the membrane for about eighteen hours, a further 150–200 c.c. was allowed to flow slowly through the cell. Before starting a measurement the solvent half cell was also flushed with a large volume of solvent. After the measurement the solution chamber was washed free of solute. The solutions used were prepared by volume dilution from a stock solution. Rate determinations were made on both sides of the equilibrium position. All measurements were made at 25°C.

A typical rate diagram is shown in Fig. 9. The interferometer was so adjusted that the extrapolated value of the reference position is zero. This adjustment was made once and for all at the beginning of the series of experiments when both half cells were filled with water. A check of the reference position after a series of measurements showed that the zero point had not changed and it was therefore not necessary to apply a correction to the observed osmotic pressures.

While the two reference lines extrapolated to the same point, they are not coincident. This effect is probably due to the difficulty of completely washing out traces of polymer in the membrane.

Ideally, all the flow lines on the rate diagram should be parallel. Within the experimental error this is true in the case of the solutions. The marked difference between the slope for the solutions on the one hand, and the solvent on the other might be explained by a diminished membrane permeability due to adsorption of polymer on the membrane.

In calculating the reduced osmotic pressures the concentrations used were those of the solutions as prepared. The effect of adsorption on the concentration was eliminated by the method of conditioning the membrane with large volumes of solution over a long period of time. Since the slopes are constant it is reasonable to assume that the adsorption was the same for each solution and that it was already complete during the initial period of conditioning. The cell contains about 7 c.c. of solution and the volume of solvent passing through the membrane in either direction during a measurement is estimated to be less than 0.03 c.c. The corresponding uncertainty in the concentration of the solution is less than 0.5 per cent and can be neglected.

One of the primary conditions for obtaining reliable osmotic pressure data is that the membrane must be strictly semipermeable. Because of the small concentrations used in the present experiments it would have been very difficult to ascertain the existence of solute permeation through the membrane by the evaporation to constant weight of solution or solvent after the measurements. Nevertheless, the good linearity of the rate diagrams indicates that there was no leakage in the measurements described here.

Lang (34) has shown that if the osmotic pressure is to be expressed in terms of solvent, but is measured as the difference between the height of a column of solvent and a column of solution, a correction has to be applied to allow for the different

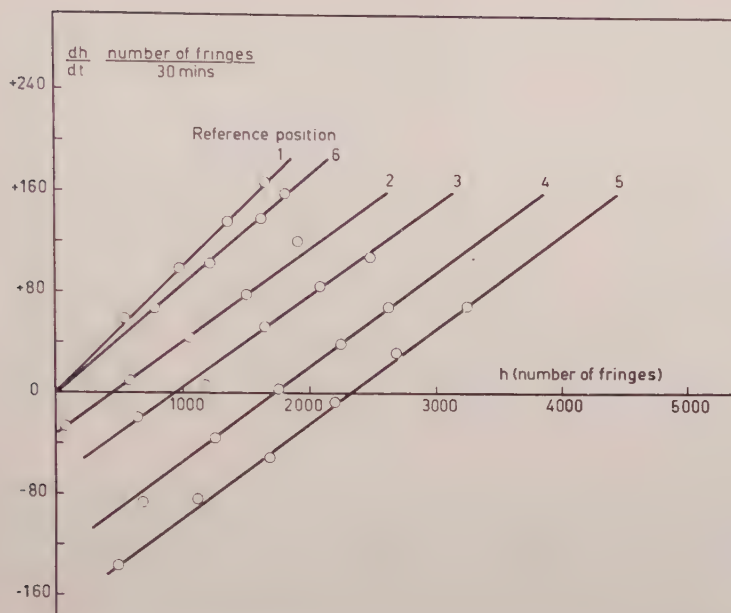


Fig. 9. Typical rate diagram showing curves for the reference position (1) before experiments, (6) after experiments, and for different concentrations of a fraction (M7) of ethyl hydroxyethyl cellulose in water. (577.6 fringes = 1 mm.)

Table 5. Osmotic pressures for aqueous solutions of ethyl hydroxyethyl cellulose at 25°C.

Fraction No.	c g/100 c.c.	π cm H ₂ O	$\pi/c \times 10^{-4}$ dyn. cm g ⁻¹	$M_n \times 10^{-3}$	$B \times 10^4$ mol. cm ³ g ⁻²
M12	0.292	0.538	18.0	176	5.45
	0.120	0.191	15.5		
	0.0700	0.107	15.1		
	0		14.0		
M7	0.173	0.403	22.8	128	7.63
	0.126	0.298	23.2		
	0.0754	0.159	20.8		
	0.0377	0.0763	19.9		
	0		19.2		
M5	0.167	0.606	35.6	77.6	9.80
	0.0791	0.270	33.5		
	0.0475	0.159	32.9		
	0		31.8		

density of the solution. The corrected osmotic pressure is given by

$$\pi = h d_c + H(d_c - d_0) \quad (13)$$

where h is the observed equilibrium height difference, H is the height of the solution column, and d_c and d_0 are the densities of the solution and solvent respectively.

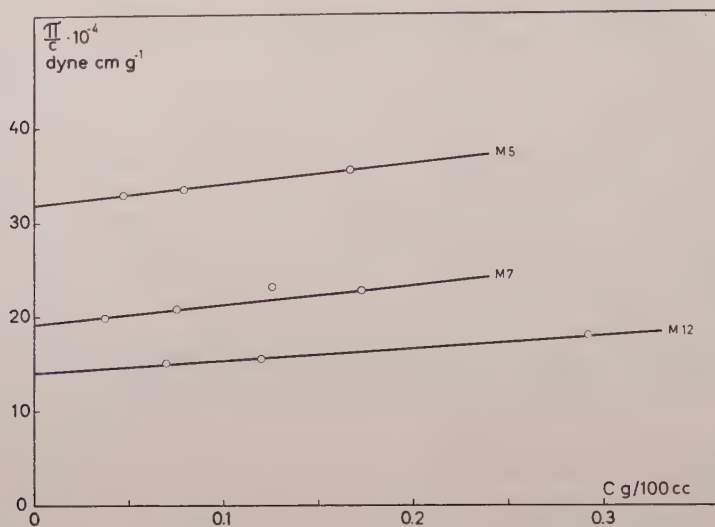


Fig. 10. Reduced osmotic pressure as a function of concentration for three fractions of ethyl hydroxyethyl cellulose in water.

In the Fuoss Mead osmometer, H is measured from the centre of the vertical membrane and in the present case is equal to 22 cms. Density measurements, to be described later, revealed that the relation between density and concentration for aqueous solution of ethyl hydroxyethyl cellulose at 25°C can be represented by the formula

$$d_c = d_0 + Kc \quad (14)$$

where K is a constant and c is expressed in g/100 c.c. From equations 13 and 14, and forming the limiting value of the reduced osmotic pressure according to equation 11 we obtain

$$\frac{RT}{M} = \lim_{c \rightarrow 0} \frac{h d_c}{c} + HK. \quad (15)$$

The correction term is thus independent of concentration and its neglect in the case of high molecular weight materials may lead to considerable error. In the present study it has been applied in all cases.

A further correction for dispersion effects (30) amounting to a maximum of about one per cent has been applied to the data.

The results obtained are collected in Table 5 and by way of illustration the reduced osmotic pressure is plotted against concentration in Fig. 10. The density correction term in equation 15 amounted to 0.5, 0.4 and 0.2 per cent of $\lim_{c \rightarrow 0} \pi/c$ for samples 12, 7 and 5 respectively. The second osmotic virial coefficients calculated from the slopes of $\pi/c = f(c)$ plots are also given in Table 5.

(5) Specific refractive index increment

A knowledge of the specific refractive index increment, dn/dc , is necessary in the experiments to be described hereafter. Measurements were made with the aid of

Table 6. Refractive index increment of fractions of ethyl hydroxyethyl cellulose in water. Wavelength 546 m μ

Fraction No.	c g/100 c.c.	$\Delta n \times 10^4$	dn/dc c.c./g
M3	0.986	14.43	0.146
M5	1.099	16.19	
	0.746	10.86	0.146
M7	0.535	8.034	
	0.799	12.024	
	1.095	16.830	0.151
M12	0.568	8.480	
	1.098	16.255	0.148

a double prism differential refractometer having an optical system similar to that of an ordinary spectrometer. The displacement of the slit image was measured with a travelling microscope, the reading accuracy being about $\pm 3 \times 10^{-7}$. During the calibration of the instrument with aqueous solutions of sodium sulphate, the reproducibility of the values was $\pm 3 \times 10^{-6}$. The refractive index increment Δn was measured at a wave-length of 546 m μ for a series of concentrations (between 0.3 and 1.1 per cent) of each fraction. The results are given in Table 6. The differences observed between the values of dn/dc are probably due to variations in the quality of the solvent. For similar ethyl hydroxyethyl celluloses Jullander reported a value of 0.146 c.c./g for dn/dc .

(6) Sedimentation

Sedimentation studies were carried out in a Svedberg oilturbine ultracentrifuge. Details concerning the construction and operation of this instrument have been given in a monograph by Svedberg and Pedersen (35).

The course of the sedimentation was followed by the Lamm scale method (36). A scale with lines ruled perpendicular to the direction of sedimentation is photographed through the cell. The concentration gradient at the sedimenting boundary produces a shift of the scale lines from their original positions on the photographic plate. This displacement, Z , is given by

$$Z = G a b \alpha dc/dx \quad (16)$$

where G is a photographic enlargement factor, a is the length of solution through which the light beam passes, b is the distance from the scale to the middle of the cell and α the refractive index increment dn/dc of the solute. Thus if the refractive index increment may be regarded as constant, and this is usually the case, the concentration may be obtained by the integration of the $Z(x)$ curve. On a $Z(x)$ curve, every sedimenting boundary is represented as a peak and the sedimentation velocity is calculated by determining the position of this peak at different times from the start of the experiment. The sedimentation constant, s , is defined by the equation

$$s = (dx/dt)/\omega^2 x \quad (17)$$

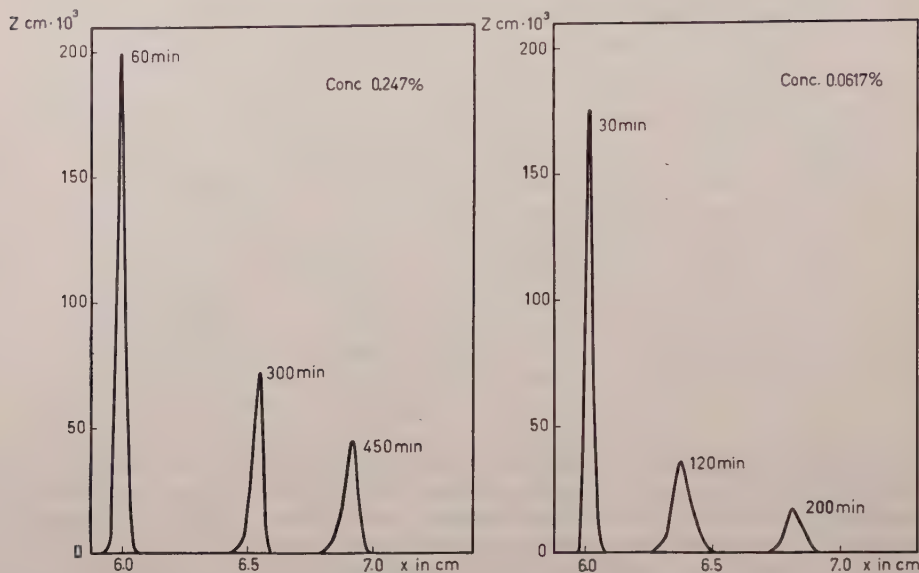


Fig. 11. Sedimentation diagrams for aqueous solutions of ethyl hydroxyethyl cellulose fraction M12. x = distance from the centre of rotation, Z = scale-line displacement. The curves to the left and right correspond to a scale distance of 10 and 40 respectively.

where x is the distance from the centre of rotation, ω is the angular velocity and t the time in seconds.

The evaluation of the sedimentation constant permits the calculation of the molecular weight by the classical Svedberg relation (35)

$$M = \frac{RTs}{D(1 - V\rho)} \quad (18)$$

where R is the gas constant, T the absolute temperature, D the diffusion constant, V the partial specific volume of the solute and ρ the density of the solvent.

As a polydisperse substance sediments in a centrifugal field, the width of the boundary increases progressively with the distance, x , of sedimentation. Gralén (37) proposed the quantity, dB/dx at zero concentration, as a relative measure of the polydispersity of the sample. B is defined as the average width of the curve and is obtained by dividing the area under the peak by its maximum height.

The sedimentation measurements were made at a speed of 1000 r.p.s., corresponding to a centrifugal force of 260,000 times gravity. The temperature of the rotor was regulated by cooling the turbine oil and the temperature was recored by means of a thermocouple placed immediately adjacent to the rotor. Measurements were generally made at a temperature of about 26°C. The sedimentation constants have been reduced to the standard temperature of 20°C according to the relation (35)

$$s_{20} = s_t \frac{\eta_t}{\eta_{20}} \frac{(1 - V_{20}\rho_{20})}{(1 - V_t\rho_t)} \quad (19)$$

where s_{20} and s_t are the sedimentation constants at 20° and t° respectively, η_{20} , η_t , and ρ_{20} , ρ_t are the viscosities and densities of the solvent at the corresponding temperatures and V is the partial specific volume of the solute.

As previously found by Jullander (8) the sedimentation curves showed well-defined and fairly symmetrical peaks. A few typical examples are shown in Fig. 11. The position of the sedimenting boundary was determined from the maximum point of the curve and the sedimentation constants were calculated from the slope of a $\ln x_t$ vs. t plot, where x_t is the position of the boundary at the time t . The values thus obtained were reduced to the standard value at 20°C , as described above.

Since the fractions used were not very sharp, the question arises as to what type of mean value s is obtained by the method of calculation used. For symmetrical sedimentation curves a weight average sedimentation constant s_w is obtained when the sedimentation velocity is calculated from the movement of the maximum point of the curve (38). It is therefore expected that the sedimentation constants reported here should lie very close to a weight average.

In a number of instances, especially at low concentrations and late exposures, there were disturbances in the sedimentation which made it impossible to obtain any significant results from the calculation of Gralén's dB/dx .

Extrapolation of the sedimentation constant to infinite dilution

The variation of the sedimentation coefficient with concentration presents a rather uncertain and complex situation. As the concentration is reduced, s increases sharply, due to decreased molecular interference. The concentration range in which spherical molecules sediment independently can usually be reached experimentally, but for thread-like molecules of the same molecular weight, s is still increasing rapidly at the lowest attainable concentrations. As there is at present no complete theory covering the dependence of s on c , the extrapolation must usually be made semi-empirically. It is then necessary to rely heavily on the s -values at the lowest concentrations and these are precisely the least accurately known ones.

Kermack *et al.* (39) and more recently Burgers (40) have given a hydrodynamic treatment of the concentration dependence of sedimentation for spherical particles in dilute solutions. They showed that the reciprocal of the sedimentation constant should increase linearly with the concentration, and this has been found to be true for a number of systems (cf. e.g. ref. (41)).

Gralén (37) has proposed the relation

$$s = s_0 (1 + Kc)^{-1}$$

where s_0 is the sedimentation constant at infinite dilution and K is a constant. He found that this equation, which leads to a linear plot between s and sc , applied rather well to his cellulose data. However, more recent work has shown that it is only of limited applicability (38).

The sedimentation constant may be related to the frictional coefficient f by means of the Svedberg equation (35)

$$s = (M/N) (1 - V\rho)/f \quad (20)$$

where N is Avogadro's number and ρ is the density of the solution. The frictional coefficient may then be represented by a power series in concentration (42), thus

Table 7. Sedimentation-concentration data for ethyl hydroxyethyl cellulose in water at 20°C.

Fraction No.	Conc. in g/dl		$s_{20} \times 10^{13}$	η/η_0	k	$-k'$	$s_0 \times 10^{13}$
	c_0	$(c)_{av}$					
M12	0.2470	0.214	1.36	7.82	2.96	4.83	3.00
	0.1650	0.144	1.56	4.12			
	0.1235	0.113	1.69	2.91			
	0.0823	0.088	1.92	2.07			
	0.0617	0.048	2.10	1.74			
	0.0463	0.036	2.34	1.50			
	0.0308	0.023	2.60	1.31			
M7	0.3058	0.308	1.25	7.10	2.70	4.94	2.63
	0.2039	0.171	1.39	3.90			
	0.1529	0.125	1.52	2.80			
	0.1019	0.093	1.76	2.02			
	0.0764	0.059	1.85	1.70			
	0.0306	0.021	2.38	—			
M5	0.2416	0.226	1.29	2.50	4.67	2.32	
	0.2013	0.174	1.32				
	0.1611	0.129	1.38				
	0.1208	0.108	1.49				
	0.0805	0.061	1.65				
	0.0403	—	1.73				
M3	0.3106	0.274	1.11	3.11	5.86	2.00	
	0.2071	0.199	1.19				
	0.1553	0.140	1.14				
	0.1035	0.084	1.31				
	0.0776	0.073	1.43				
	0.0444	—	1.60				

$$f = f_0 (1 + kc + k'c^2 + \dots) \quad (21)$$

where k and k' are constants characterizing the concentration dependence of the frictional coefficient. From equations (20) and (21) the sedimentation constant is therefore given by

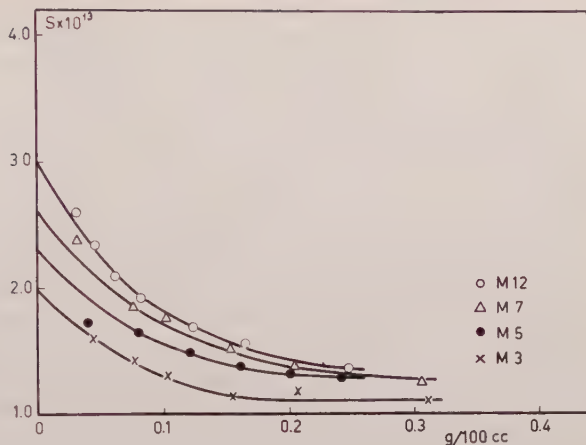
$$s = s_0 (1 + kc + k'c^2)^{-1}. \quad (22)$$

Newman *et al.* (43) found that such an equation gave a very accurate representation of their data for cellulose nitrate in acetone over a fairly wide concentration range. They determined the constants graphically by a difference method from the relation

$$(1/s_2 - 1/s_1)/(c_2 - c_1) = k + k'(c_1 + c_2) \quad (23)$$

where the subscripts 1 and 2 refer to any pair of data. Following this, s_0 was evaluated graphically from plots of $(1/s - k'c^2)$ vs. c .

Fig. 12. Variation of sedimentation constant with concentration for fractions of ethyl hydroxyethyl cellulose in water.



Lauffer (44) has shown that the variation of the sedimentation constant with concentration can be corrected for the viscosity of the solution by the factor $s\eta/\eta_0$. This latter expression should be independent of the concentration of the solution. A similar conclusion was reached by Powell and Eyring (45). This seems to work fairly well with proteins and certain synthetic polymers but in most cases leads to an overcorrection, the corrected sedimentation constant increasing with concentration. The method is nevertheless interesting from another point of view. Since $\lim_{c \rightarrow 0} s$ must be identical with $\lim_{c \rightarrow 0} s\eta/\eta_0$, the plotting of both $s\eta/\eta_0 = f(c)$ and $s = f(c)$ on the same diagram may serve to reduce the uncertainties in the extrapolation procedure (38).

It has been pointed out by a number of authors that in determining the relationship between s and c , the value of c to be used is the average value of the concentration at the sedimenting boundary and not the concentration of the solution prepared for the run. Because of the sector-shape of the ultracentrifuge cell and the change of centrifugal force with distance from centre of rotation, the concentration at any cross-section of the sedimenting column diminishes during the run in accordance with the formula (ref. 35)

$$c_t = c_0(x_0/x_t)^2$$

where t denotes the time from the beginning of the sedimentation. Concentrations, as determined by measuring the areas under the peaks and correcting for dilution effects as indicated above, were generally 10–25 per cent less than the original concentrations. There were considerable difficulties in knowing where to place the base lines of the curves and since furthermore the peaks were very sharp and narrow, the differences noted are certainly within the uncertainty in the area under the peaks. For this reason the original concentrations were corrected for the dilution effect and used in determining the relationship between s and c . Both concentrations are given in Table 7 where also the sedimentation constants of the various fractions are summarized. For fractions M12 and M7 the relative viscosities, measured as previously described, at each concentration are also given in the table.

Fig. 12 shows a plot of s against c for each fraction. It is apparent that the con-

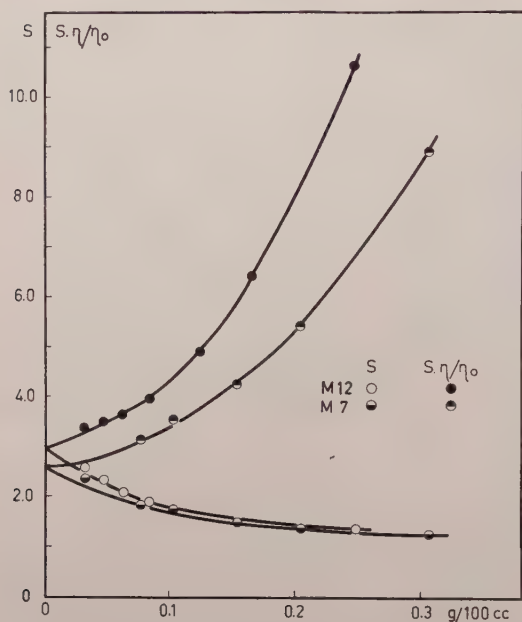


Fig. 13. Sedimentation constant s , and $s\eta/\eta_0$ as a function of concentration, c , for two fractions of ethyl hydroxyethyl cellulose in water. s in Svedberg units.

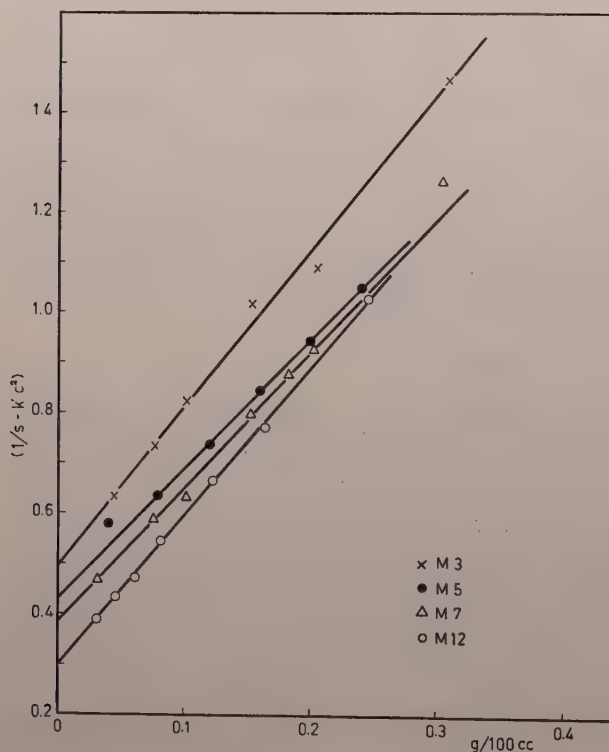


Fig. 14. Linear extrapolation of sedimentation constants to infinite dilution for fractions of ethyl hydroxyethyl cellulose in water.

centration dependence of s is rather marked. For the three highest fractions already at a concentration of 0.2 per cent the sedimentation constants are almost identical and it is clear that above this concentration no free sedimentation can occur.

Such effects have previously been observed (38, 41, 46) and may be explained by assuming that the more slowly sedimenting molecules hinder the motion of the larger ones and completely dominate the observed sedimentation rate at higher concentrations (46).

It is also evident that the sedimentation velocity does not differ markedly from one fraction to another. The molecular weight of fraction M12 is about three times greater than that of M3, yet the sedimentation velocities differ by only fifty per cent. This slight dependence of the velocity on the molecular weight has also been observed for another water soluble cellulose ether, namely methyl cellulose (5).

One could not be sure that the curves would not bend up sharply at the lower concentrations where it was impractical to carry out measurements. However, by plotting $s\eta/\eta_0 = f(c)$ and $s = f(c)$ on the same diagram some of the uncertainty in the extrapolation procedure was removed. An example is given in Fig. 13.

While plots of $1/s$ against c were non-linear, the quadratic equation was found to represent the data rather well and yielded values of s_0 in close agreement with those of the former procedure. Fig. 14 shows the results obtained by this method of extrapolation. The constants k and k' were determined as mentioned above.

(7) Diffusion

The diffusion experiments were carried out with the aid of the Lamm scale method (cf. page 537). When the scale line displacement Z is plotted against the scale line position x , a frequency curve is obtained which in the case of a monodisperse substance diffusing from an initially sharp boundary between solution and solvent conforms to Wiener's equation (47)

$$\frac{dn}{dx} = \frac{n_1 - n_0}{2\sqrt{\pi Dt}} e^{-x^2/4Dt} \quad (24)$$

Here D is the diffusion constant, x the distance from the original boundary, t the time after the beginning of the diffusion, and $n_1 - n_0$ is the difference in refractive index between solution and solvent. The scale method of Lamm gives dn/dx as a function of x . From equation (24) a number of formulae have been derived for the calculation of D (36).

In the case of a polydisperse substance the calculation of the different mean values of the diffusion coefficient from the experimentally obtained diffusion curve is based upon the following formulae given by Lamm (36) and Gralén (37)

$$D_A = A^2/4\pi t H \quad (25)$$

$$D_m = m_2/2At \quad (26)$$

where A is the area between the diffusion curve $dn/dx = f(x)$, and the x axis, H is the maximum height of the curve, and m_2 is the second moment around the vertical axis through the arithmetic mean of the curve. D_A and D_m are denoted as the diffusion coefficient according to the "area" and moment method respectively.

The physical significance of these two diffusion constants has been discussed in detail by Gralén (37). For a polydisperse substance D_A represents a mean value which lies between a number and a weight average, while D_m is a weight average of the different constants of the constituents. Theoretically, for a polydisperse substance $D_m > D_A$ and the ratio D_m/D_A , equal to unity for a monodisperse substance, has been proposed by Gralén (37) as a measure of polydispersity. However, as he has also pointed out, a very high accuracy is necessary for this calculation. Due caution must therefore be exercised in applying this method.

It is often advantageous to transform the arbitrary coordinates of the experimentally observed diffusion curve into normal coordinates (normal) curve as a means of comparing the experimental curves referring to the different times of diffusion in the same dimensionless units, or of comparing an experimental curve with the corresponding ideal Gaussian curve. This is ordinarily accomplished by the transformations

$$\xi = x/2\sigma \quad \text{and} \quad \psi = \frac{5\sigma}{\Phi} \left(\frac{dn}{dx} \right) \alpha \quad (27)$$

where α is a constant of the optical system, Φ is the area of the (dn/dx) versus x curve, $\sigma = \sqrt{2Dt}$ and 2 and 5 are arbitrary constants chosen for convenience in plotting. The ideal curve, which may be plotted from mathematical tables, has the form

$$\psi = \frac{5}{\sqrt{2\pi}} e^{-2\xi^2} \quad (28)$$

The methods of carrying out this calculation have been discussed by Beckmann and Rosenberg (48).

Deviations from ideal behaviour in the experimental curves may be due to polydispersity in the sample or to a dependence of the diffusion rate on concentration. Lamm (36) has shown that polydispersity causes the ordinates of the normal curve in the vicinity of $x=0$ to be higher than those of the ideal Gaussian curve. A concentration dependence of the diffusion rate reveals itself as a lateral deviation of the experimental normalized curve from the ideal. Qualitatively, a positive concentration dependence is manifested by a displacement or skewness of the experimental curve toward the solvent side of the original boundary position, while curves with a negative concentration dependence are displaced toward the solution side.

Experimental methods and results

The diffusion measurements were carried out in two different cells which made possible the formation of a very sharp and plain boundary between solution and solvent. One of these cells was of the flat Claesson type (49), while the other was of a type developed by Claesson *et al.* during an extensive investigation of methods of high precision diffusion. This cell differs from the original design in that the upper half of the diffusion chamber forms part of a round block which can be moved into coincidence with the lower half of the chamber by means of a worm gear. Fig. 15 shows a photograph of the cell. Full details will be given elsewhere. As a check on the overall validity of the experimental technique measurements were carried out

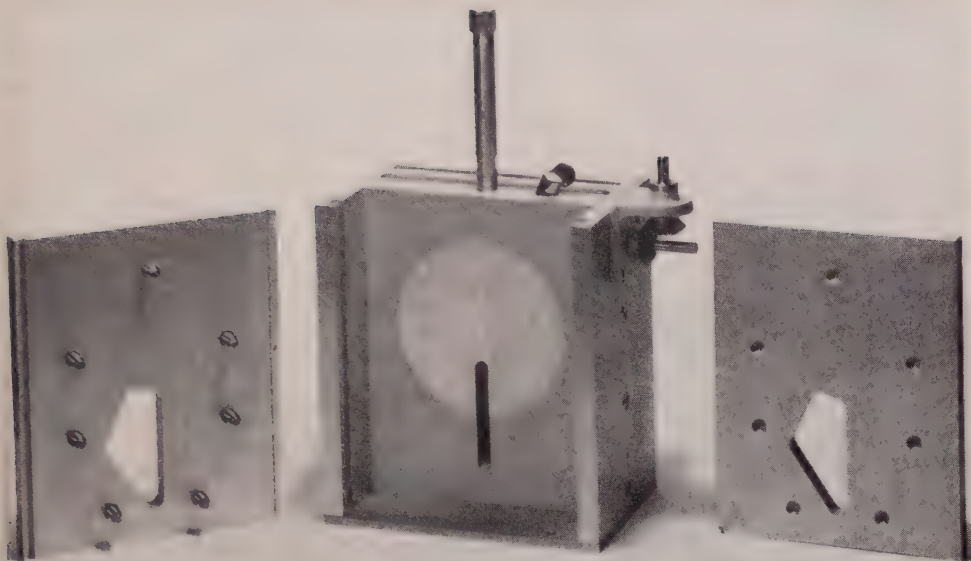


Fig. 15. Photograph of the diffusion cell with circular sliding surfaces.

with sucrose solutions. Gosting and Morris (50) have reported a value of $D_{24.90} = 51.48 \times 10^{-7} \text{ cm}^2/\text{sec.}$ determined by the Gouy interference technique. When corrected to 20° this value becomes $45.07 \times 10^{-7} \text{ cm}^2/\text{sec.}$ With both cells this value was reproduced within 3 per cent. Best results were obtained when the sliding surfaces were lubricated with high quality silicone grease.

The measurements were carried out at a temperature of $22.0 \pm 0.01^\circ\text{C}$ for at least four concentrations of each fraction. Since the diffusion constant depends upon the viscosity of the medium the observed values were reduced to the standard temperature of 20°C by means of the formula

$$D_{20} = D_t \frac{\eta_t}{\eta_{20}} \frac{293}{T} \quad (29)$$

where D_{20} is the diffusion coefficient in water at 20°C , D_t the observed diffusion coefficient and η_t/η_{20} the ratio of the viscosity of the solution investigated to that of pure water at 20°C .

Great care was taken to have thermal equilibrium between the liquids in the two chambers of the cell before forming the boundary. Exposures were taken at intervals of about three hours over a period of 50–75 hours depending on the concentration of the solution. The measurements on fraction M7 were carried out in the flat cell and all other measurements were performed in the round cell.

The values of D_A and D_m were calculated from the slopes of plots of A^2/H^2 and m_2/A respectively as a function of time. These plots were linear and intercepted the t -axis at or close to the origin, thereby suggesting that the experiments had proceeded undisturbed and without delay and also that sharp boundaries had been formed between solution and solvent.

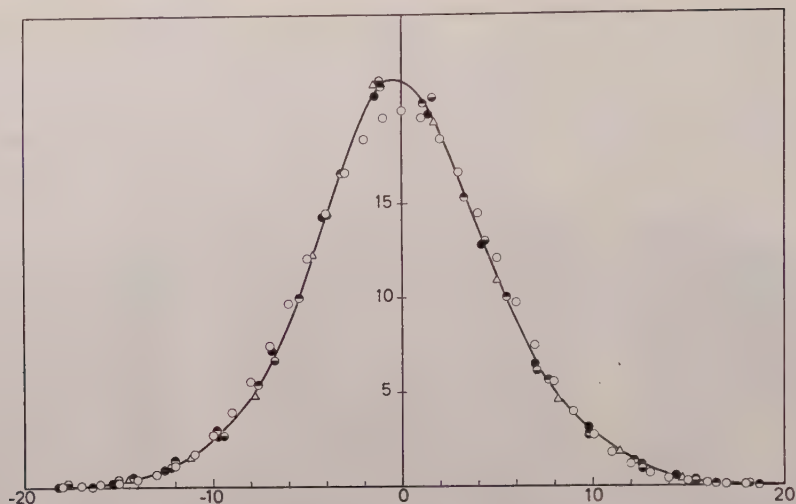


Fig. 16. The diffusion curve in normal coordinates of ethyl hydroxyethyl cellulose fraction M3 in water. The curve connects the experimental points obtained from different exposures. The open circles indicate the corresponding normal curve.

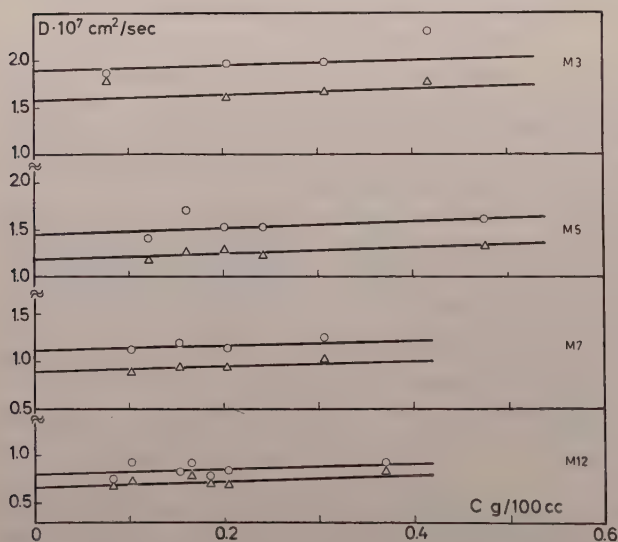


Fig. 17. Concentration dependence of D_A (triangles) and D_m (circles) for four fractions of ethyl hydroxyethyl cellulose in water.

Table 8 gives the results of the determinations. The values are given in units of cm^2/sec . In Fig. 16 the normal diffusion curve for one of the fractions is compared with the corresponding ideal Gaussian curve. The slight skewness of the experimental curve towards the solvent side indicates a small positive concentration dependence. In agreement with theory the values of D_m are in all cases higher than D_A and the rather high D_m/D_A values (see Table 8) may be taken as an indication that the fractions are quite polydisperse.

Table 8. Diffusion constants for different fractions of ethyl hydroxyethyl cellulose in water at 20°C. Concentration in g/100 c.c.

Fraction No.	Conc.	$D_m \times 10^7$	$D_A \times 10^7$	D_m/D_A	$k_D \times 10^7$
M3	0.417	2.30	1.76	1.31	0.3
	0.308	1.98	1.66	1.19	
	0.205	1.97	1.60	1.23	
	0.078	1.87	1.78	1.05	
	0	1.9	1.6	1.20	
M5	0.475	1.60	1.32	1.25	0.3
	0.242	1.52	1.22	1.24	
	0.201	1.53	1.29	1.19	
	0.161	1.70	1.26	1.35	
	0.121	1.40	1.17	1.20	
M7	0	1.4	1.2	1.2	0.3
	0.102	1.12	0.88	1.27	
	0.153	1.19	0.93	1.28	
	0.204	1.13	0.94	1.20	
	0.306	1.24	1.02	1.21	
M12	0	1.1	0.90	1.2	0.3
	0.370	0.92	0.83	1.11	
	0.204	0.83	0.68	1.22	
	0.185	0.77	0.69	1.12	
	0.165	0.91	0.77	1.18	
	0.153	0.82	0.79	1.04	0.3
	0.102	0.92	0.72	1.28	
	0.082	0.74	0.66	1.12	
	0	0.8	0.6	1.3	

It is often found that the concentration dependence of the diffusion constant may be represented by the linear equation

$$D = D_0(1 + K_D c) \quad (30)$$

where D and D_0 are respectively the diffusion constant at any concentration c and at infinite dilution and K_D is an empirical constant. By assuming such a linear dependence Gralén (37) has shown that the value of D_m may be corrected to zero concentration by the equations

$$D_m = D_0 \left(1 + \frac{K'_D c}{2} \right) \quad (31)$$

$$D_0 = D_m + \frac{A M_0}{4 H T} \quad (32)$$

where K'_D is a constant independent of molecular weight and M_0 is the displacement of the maximum ordinate at time t . The use of these equations is considered to be theoretically unsound in the present case due to the previously mentioned necessity of including a quadratic concentration term in the concentration dependence of the frictional coefficient.

In all cases the diffusion constant was a linear function of concentration thus permitting graphical extrapolation to infinite dilution. In Fig. 17 both D_A and D_m are plotted against concentration.

Within the experimental error all the curves have the same slope. The value of K_D in equation 30 is given in Table 8.

(8) Partial specific volume

In order to calculate molecular weights from sedimentation and diffusion data, it is necessary to know the partial specific volume of the substance in solution. This was determined from density measurements on solutions of each of the fractions studied. The measurements were made at $20 \pm 0.01^\circ\text{C}$ and in the concentration range 0.3–2 per cent. The pycnometer used was a cylindrical glass vessel holding about 30 c.c. The neck was made of capillary tubing of about 1 mm diameter and a well-fitting ground-glass cap was placed over the exit. During the filling, which was accomplished by means of a syringe, air bubbles were easily formed and had to be carefully removed before weighing. Buoyance corrections were applied throughout.

The solutions used were prepared by dilution from a stock solution from which traces of salt had been removed by electrodialysis. The concentration of each solution was determined by the method previously described (see page 526).

The partial specific volume was calculated from the formula

$$V = v \left(1 - \frac{1-p}{m} \frac{dm}{dp} \right) \quad (33)$$

(cf. Kraemer in ref. (35)) where v is the specific volume of the solution, m is the weight of the pycnometer content, and p the concentration of the solution expressed as a weight fraction. dm/dp was determined by the method of least squares from the slope of an m vs. p plot. In all cases this function was linear. The complete results with weighted averages are given in Table 9.

(9) Molecular size and shape from sedimentation-diffusion

Molecular weights, $\bar{M}_{wv}$ (weight-weight average) were calculated from the sedimentation-diffusion measurements according to equation (18) using the extrapolated values of s and D_m given in Tables 7 and 8, and the partial specific volumes of Table 9. The results are given in Table 10. As previously pointed out the values of s_0 and D_0 are close to weight averages and it should be noted that the molecular weight thus obtained is, according to Jullander (38), somewhat less than a true weight average. It is common experience that the most serious limiting factor in the determination of molecular weight by this method is the uncertainty in the value of D_0 . In the case of sample M12 this is especially true since the diffusion constants are so low that a small error in the extrapolation may lead to a considerable error in the molecular weight. Nevertheless, as will be seen later, the values obtained here are in substantial agreement with the closely related weight average given by another method and are therefore probably correct within about 10 per cent.

From the sedimentation and diffusion measurements it is possible to derive some information about the shape and dimensions of the molecules in solution (35).

Table 9. Partial specific volume of ethyl hydroxyethyl cellulose in water at 20°C.

Fraction No.	$p \times 10^3$	m	v	V
M12	0	29.0603	1.0018	0.7053
	0.5674	29.1085	0.9983	0.7050
	1.0950	29.1545	0.9968	0.7059
Mean				0.705
M7	0	29.0603	1.0018	0.7034
	0.3055	29.0864	0.9991	0.7027
	0.5342	29.1069	0.9984	0.7031
	0.7974	29.1310	0.9976	0.7035
	1.0915	29.1531	0.9968	0.7041
	1.4561	29.1863	0.9957	0.7047
Mean				0.703
M5	0	29.0603	1.0018	0.7105
	0.4907	29.1011	0.9986	0.7101
	0.7445	29.1216	0.9979	0.7105
	1.0906	29.1525	0.9968	0.7110
	1.4590	29.1835	0.9958	0.7117
Mean				0.711
M3	0	29.0603	1.0018	0.7210
	0.3106	29.0845	0.9992	0.7202
	0.6536	29.1132	0.9982	0.7207
	0.9838	29.1384	0.9973	0.7213
	1.4253	29.1756	0.9960	0.7219
	1.9186	29.2165	0.9946	0.7226
Mean				0.721

Table 10. Molecular constants of ethyl hydroxyethyl cellulose as derived from sedimentation-diffusion.

Fraction No.	$M \times 10^{-3}$	f/f_0	l/d	$\frac{l}{A}$	$\frac{d}{A}$	$l_{\max}$	$l/l_{\max}$
M12	310	6.0	220	3300	15	7600	0.43
M7	200	5.1	160	2200	14	4900	0.45
M5	140	4.1	100	1500	15	3400	0.44
M3	92	3.8	86	1100	13	2200	0.50

For spherical unhydrated particles of molecular weight M , the molar frictional coefficient f_0 calculated from Stokes's law is given by

$$f_0 = 6\pi\eta N \left(\frac{3MV}{4\pi N} \right)^{\frac{1}{3}} \quad (34)$$

where η is the viscosity of the solvent and the other symbols are as previously

defined. On the other hand the frictional coefficient may also be calculated from the sedimentation and diffusion constant according to the expression

$$f = \frac{RT}{D} = \frac{M(1 - V\rho)}{s} \quad (35)$$

If the experimental values are reduced to the basis of water at 20°C, equations 34 and 35 may be combined to yield

$$f/f_0 = 10^{-8} \left(\frac{1 - V\rho}{D_{20}^2 s_{20} V} \right)^{\frac{1}{2}} \quad (36)$$

When the frictional ratio f/f_0 is equal to unity it indicates that the molecules are spherical and unhydrated. On the other hand if $f > f_0$ it implies molecular hydration, asymmetrical molecules or a combination of both factors.

Herzog, Illig and Kudar (51) have shown that, assuming the molecule to be unhydrated and to have the shape of a prolate spheroid of length l and thickness d , the frictional ratio is related to the axial ratio l/d by means of the equation

$$f/f_0 = \frac{\sqrt{1 - p^2}}{p^{\frac{2}{3}} \log \left(\frac{1 + \sqrt{1 - p^2}}{p} \right)} \quad (37)$$

Svedberg and Pedersen (35) and Gralén (37) have given tables for f/f_0 as a function of l/d .

The length and diameter of the assumed ellipsoid are related to the molecular weight and partial specific volume by the formula

$$\frac{\pi l d^2}{6} = \frac{MV}{N} \quad (38)$$

From this and the value of l/d , the absolute values of l and d may be evaluated. Taking the length of the polymerizing unit, that is the glucose residue, to be 5.15 Å, the length of a fully stretched molecule can be calculated from the degree of polymerization Z according to the relation

$$l_{\max} = 5.15 Z.$$

Values of f/f_0 , l/d , l and d calculated from these relationships are given in Table 10. In the absence of a knowledge of the state of hydration of the molecules in solution the only definite conclusion which may be drawn is that the molecules are non-spherical. However, as it is unrealistic to believe that the strong deviations from spherical shape are due in any marked degree to molecular hydration, we may tentatively assume that the molecules are strongly asymmetrical. A comparison of l and $l_{\max}$ indicates that in solution the molecules are coiled to about $\frac{1}{2}$ of their fully extended length. It must be emphasised that the application of this model is highly questionable in the present case and the dimensions given must therefore be taken with considerable reservation. Other methods which give a more accurate and unambiguous description of the state of the molecule in solution will be considered later.

(10) Sedimentation equilibrium

When a solution of a high-molecular substance is subjected to a low centrifugal field a state of equilibrium may finally be established in which, for every level in the cell, the rate at which the molecules are sedimented outwards is balanced by the rate at which they diffuse inwards. From a knowledge of the concentration distribution in the cell at equilibrium the molecular weight may be computed according to the formula

$$M = \frac{2RT \log (c_2/c_1)}{(1 - V\rho)\omega^2(x_2^2 - x_1^2)} = \frac{RT (dc_x/dx)}{(1 - V\rho)\omega^2 x c_x} \quad (39)$$

where c_1 , c_2 and c_x are respectively the concentrations at the distances x_1 , x_2 and x from the centre of rotation and the other symbols have the previously stated significance.

When, as in the present case, the Lamm scale method is used to determine the variation of concentration with distance in the cell, it is necessary to convert the scale line displacement (Z) versus distance (x) curve to a concentration versus distance curve in order to apply equation (39) directly. However, the molecular weight may also be calculated from the alternate formula

$$M = \frac{2RT \ln Z_2 x_1 / Z_1 x_2}{(1 - V\rho)\omega^2(x_2^2 - x_1^2)} \quad (40)$$

where Z_1 and Z_2 are the scale line displacements at the points x_1 and x_2 respectively.

The above equations give the true molecular weight only for substances which are monodisperse and whose solutions exhibit ideal thermodynamic behavior.

When the system is polydisperse each molecular species sets up its own equilibrium independently of the others and thus the values of M obtained vary with the position x in the cell. Under these circumstances a weight average value of the molecular weight, $\bar{M}_w$, for the whole sample may be obtained from the expression

$$\bar{M}_w = \frac{\int M_{wx} c_x x dx}{\int c_x x dx} \quad (41)$$

where M_{wx} is calculated from equation 39 in its differential form and where the summation is extended over the entire length of the solution in the cell.

It is also possible to construct the so-called Z -average molecular weight defined by

$$\bar{M}_z = \frac{\sum_i w_i M_i^2}{\sum_i w_i M_i} \quad (42)$$

where w_i is the total weight of molecules of molecular weight M_i . However, as has been pointed out by Williams *et al.* (52), values of $\bar{M}_z$ are subject to a considerably higher degree of uncertainty as compared with $\bar{M}_w$ since they represent a higher moment of the distribution curve. Values of $\bar{M}_z$ are useful as a means of characterizing the polydispersity of the sample. For polydisperse substances $\bar{M}_w < \bar{M}_z$.

Table 11. Sedimentation equilibrium of ethyl hydroxyethyl cellulose fraction M12 in water.

r.p.m. (scale distance)	$c_0 = 0.247\%$					$c_0 = 0.165\%$				
	x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
5,100 90	4.57	95	0.171	128,000	11,100	5.27	69	0.113	122,000	134,000
	4.62	97	0.191	116,000	11,000	5.32	80	0.128	124,000	136,000
	4.67	99	0.211	106,000	31,300	5.37	93	0.146	125,000	142,000
	4.72	103	0.232	99,000	59,900	5.42	109	0.167	127,000	128,000
	4.77	110	0.254	96,000	91,100	5.47	126	0.191	127,000	116,000
	4.82	121	0.277	96,000	104,700	5.52	144	0.218	126,000	133,000
	4.87	135	0.304	96,000	127,000	5.54	—	0.231	126,000	—
	4.92	—	0.333	99,000	—	—	—	—	—	—
Average				101,000	68,400				126,000	130,000

Table 12. Sedimentation equilibrium of ethyl hydroxyethyl cellulose fraction M7 in water.

r.p.m. (scale distance)	$c_0 = 0.201\%$					$c_0 = 0.101\%$				
	x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
5,150 120	5.16	50	0.138	103,000	—	5.78	31	0.063	122,000	—
	5.21	53	0.152	97,000	42,000	5.83	32	0.071	112,000	37,000
	5.26	56	0.166	93,000	49,000	5.88	37	0.080	114,000	129,000
	5.31	62	0.181	94,000	99,000	5.93	45	0.090	121,000	175,000
	5.36	69	0.198	95,000	111,000	5.98	56	0.103	130,000	189,000
	5.41	80	0.217	99,000	137,000	6.03	69	0.119	137,000	188,000
	5.46	93	0.240	103,000	146,000	6.08	87	0.139	147,000	208,000
	5.51	111	0.266	110,000	172,000	6.12	105	0.159	155,000	209,000
	5.54	125	0.284	116,000	198,000	—	—	—	—	—
Average				100,000	125,000				132,000	177,000

In practice macromolecular solutions are never ideal in behavior and Schulz (53) and Wales *et al.* (54) have shown that a non-ideality correction may be made by means of the relation

$$\frac{1}{M_{w_i}} = \frac{1}{M_w} + B_e c_0 \quad (43)$$

where M_w is the true weight average molecular weight, M_{w_i} is the weight average molecular weight calculated by assuming ideal behavior ($B_e = 0$) and c_0 is the initial concentration. Thus, on plotting $1/M_{w_i}$ as a function of concentration a straight with slope B_e is obtained. The intercept is the reciprocal of the true weight average molecular weight.

Wales (54, 55) has shown that the non-ideality correction factor B_e is equal to two times the osmotic second virial coefficient as defined on page 532.

Table 13. Sedimentation equilibrium of ethyl hydroxyethyl cellulose fraction M5 in water.

r.p.m. (scale distance)	$c_0 = 0.242\%$					$c_0 = 0.121\%$				
	x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
5,320 90	4.52	45	0.182	76,700	—	5.73	26	0.081	77,000	—
	4.57	48	0.194	76,000	64,000	5.78	28	0.088	75,600	62,000
	4.62	51	0.207	74,800	59,000	5.83	31	0.096	76,500	87,000
	4.67	56	0.221	76,100	96,000	5.88	38	0.105	84,700	180,000
	4.72	61	0.236	76,800	87,000	5.93	46	0.115	92,900	167,000
	4.77	68	0.253	79,000	112,000	5.98	56	0.128	100,900	171,000
	4.82	77	0.271	82,700	129,000	6.03	69	0.144	109,500	181,000
	4.87	94	0.294	92,100	211,000	6.08	90	0.164	124,400	231,000
	4.92	124	0.322	109,800	295,000	6.12	109	0.185	132,500	205,000
Average				82,800	153,000				100,000	178,000
5,270 90	4.52	28	0.187	63,000	—	5.73	16	0.086	61,000	—
	4.57	31	0.197	65,600	110,000	5.78	18	0.092	63,300	104,000
	4.62	35	0.208	69,500	133,000	5.83	21	0.099	67,900	139,000
	4.67	40	0.221	73,800	146,000	5.88	25	0.107	74,500	156,000
	4.72	44	0.236	75,300	99,000	5.93	29	0.116	79,000	131,000
	4.77	49	0.252	77,600	113,000	5.98	36	0.127	88,600	193,000
	4.82	56	0.270	82,000	142,000	6.03	47	0.141	103,500	238,000
	4.87	67	0.291	90,100	192,000	6.08	63	0.160	121,100	259,000
	4.92	77	0.316	94,400	146,000	6.12	77	0.179	131,500	219,000
Average				78,200	140,000				90,800	200,000
6,040 120	4.52	49	0.169	69,700	—	5.76	32	0.067	89,000	—
	4.37	55	0.182	71,800	96,000	5.81	42	0.077	100,000	191,000
	4.62	63	0.197	75,100	114,000	5.86	51	0.088	105,000	133,000
	4.67	70	0.215	75,600	85,000	5.91	61	0.103	107,000	122,000
	4.72	80	0.234	78,600	110,000	5.96	74	0.120	110,000	131,000
	4.77	89	0.256	79,200	85,000	6.01	90	0.141	113,000	131,000
	4.82	100	0.280	80,500	93,000	6.06	110	0.166	117,000	134,000
	4.87	112	0.308	81,100	89,000	6.11	136	0.197	120,000	140,000
	4.92	126	0.338	82,200	93,000	6.12	139	0.204	119,000	68,000
Average				78,000	95,000				111,000	134,000

Experimental and results

Sedimentation equilibrium measurements were carried out on samples M12, M7 and M5 using a Svedberg equilibrium ultracentrifuge with direct motor drive. For a description of experimental details the reader is referred to the monograph by Svedberg and Pedersen (35). Difficulties were encountered in the performance of the experiments due to the low sedimentation and diffusion constants. All experiments were made at a temperature of 20°C and in the concentration range between 0.1–0.3 per cent. Below 0.1 per cent the data obtained was unreliable while above 0.3 per cent impractically long times were required for the establishment of equilibrium. In one experiment where a concentration higher than 0.3 per cent was used, equilibrium was not established even after 2½ months. The results obtained are given in Tables 11, 12 and 13. Several other experiments which were spoilt by leak-

Table 14. Weight-average molecular weights for three fractions of ethyl hydroxyethyl cellulose in water.

Fraction No.	$\overline{M}_w$	$B \times 10^4 \text{ mole cm}^3 \text{ g}^{-2}$	
		From sedimentation	From osmotic pressure
M12	270,000	13	5.4
M7	200,000	12	7.6
M5	140,000	11	9.8

age are omitted. In the experiments reported here the time required for equilibrium was about two weeks.

Table 14 shows the true weight average molecular weights calculated according to equation (43) from the data in Tables 11, 12 and 13. It must be pointed out that the non-ideality correction has been made on the basis of measurements at two concentrations only and for this reason the final results should be considered with reservation. A justification for a linear extrapolation through two points is found in the fact that the osmotic pressure and light scattering measurements (to be described shortly) showed that the second virial coefficient was sufficient to characterise the data in the concentration range employed. Nevertheless, a comparison with the molecular weights obtained from sedimentation velocity and diffusion measurements (see Table 10) shows remarkably good agreement in all cases. It therefore seems justified to conclude that, in spite of the gross uncertainty in the non-ideality correction, the weight average molecular weights obtained in these experiments are substantially correct.

In connection with the Z -average molecular weights it will be noted that the results for fraction M12 are obviously incorrect. The accuracy of the calculation of $(M_i)_Z$ depends upon the difference between successive values of Z . Since the present system is such that these differences are low, the values of M_Z obtained cannot be relied upon. As pointed out by Svedberg and Pedersen (ref. 35, p. 315) small errors in the determination of Z will have a much larger effect on M_{Zx} than on M_{wx} .

In Table 14 a comparison is made between the second virial coefficient derived from the present experiments and that obtained directly from osmotic pressure measurements. It is seen that for samples M12 and M7 there is a considerable discrepancy for which no explanation can be offered at the present time.

(11) Light scattering

Introductory discussion

The subject of light scattering by solutions of macromolecules has been extensively reviewed by a number of authors (56–59). The following remarks are intended to summarise the theoretical basis of the method.

When a beam of unpolarised light of intensity I_0 enters a scattering medium, the intensity of the light scattered per unit volume exhibits an angular dependence given by

$$i_\theta = i_0 (1 + \cos^2 \theta) / 2 = R_\theta I_0 / r^2 \quad (44)$$

where θ is the scattering angle with reference to the forward direction of the incident beam, i_0 is the intensity of the light scattered at 0° , r is the distance between the scattering solution and the detector, I_0 is the intensity of the incident light, and R_θ is known as the reduced intensity or Raleigh ratio at the angle θ . The turbidity, τ , of the system is defined as the relative decrease of the light intensity per unit length of the primary beam as a result of scattering:

$$\tau = -dI/I dl \quad (45)$$

and is related to R_θ and i_θ by the equation

$$\tau = (2\pi r^2/I_0) \int_0^\pi i_\theta \sin \theta d\theta = 2\pi \int_0^\pi R_\theta \sin \theta d\theta. \quad (46)$$

Zernike (60) has shown that the turbidity may be written as the sum of two terms, one due to density fluctuations (solvent scattering) and the other due to fluctuations in concentration. In practice the effect of the former is eliminated by subtracting the scattering due to the solvent from that of the solution.

Debye (61) has shown that in the case of molecules whose dimensions are much smaller than about one-twentieth of the wavelength, λ , of the incident light, the turbidity due to concentration fluctuations is related to the molecular weight of the solute by the equation

$$\frac{Hc}{\tau} = \frac{1}{M} + 2Bc \quad (47)$$

where c is the concentration of polymer in solution in gm/cc, M the molecular weight of the polymer, and B the second osmotic virial coefficient (see page 532). H is a constant given by

$$H = 32\pi^3 n_0^2 \left(\frac{n - n_0}{c} \right)^2 / 3\lambda^4 N \quad (48)$$

where N is Avogadro's number, λ the wavelength in vacuo of the light used, n and n_0 are the refractive indices of the solute and solvent respectively.

Introducing instead of H , a new constant K defined by the equation

$$K = 2\pi^2 n^2 (dn/dc)^2 / \lambda^4 N, \quad (49)$$

equation (47) can be expressed in the alternate form

$$\frac{Kc}{R_{90}} = \frac{1}{M} + 2Bc. \quad (50)$$

Molecular weights are obtained by extrapolation of a plot of Hc/τ or Kc/R_{90} against c to infinite dilution. If the measurements have not been made at too high concentration the extrapolation is linear and the intercept at $c = 0$ is equal to the reciprocal of the molecular weight.

There are, however, many macromolecules having dimensions comparable to the wavelength of the incident light. As a result of the phase shifts encountered by rays scattered from different parts of the same molecule, interference effects are produced

and lead to an unsymmetrical scattering which can be characterized by a dissymmetry coefficient, z_{45} , equal to the ratio of the light scattered at 45° to the incident beam to that at 135° .

Zimm (62) has shown that in the case of large molecules equation 50 can be rewritten as

$$\frac{Kc}{R_\theta} = \frac{1}{MP(\theta)} + 2Bc \quad (0 < \theta < 180) \quad (51)$$

where $P(\theta)$ is a particle scattering factor which corrects for the effects of internal interference. $P(\theta)$ is related to the form and dimensions of the solute molecules by the following expressions (63-66)

$$\text{sphere, } P(\theta) = \left[\frac{3}{x^3} (\sin x - x \cos x) \right]^2; \quad x = \frac{ksD}{2} \quad (52)$$

$$\text{rod, } P(\theta) = \frac{1}{x} \int_0^{2x} \frac{\sin \omega}{\omega} d\omega - \left(\frac{\sin x}{x} \right)^2; \quad x = \frac{ksL}{2} \quad (53)$$

$$\text{Gaussian coil, } P(\theta) = \frac{2}{x^2} [e^{-x} + (1-x)]; \quad x = \frac{k^2 s^2 R^2}{6} \quad (54)$$

where $k = 2\pi/\lambda'$, λ' being the wavelength of light in the solution, $s = 2 \sin \theta/2$, D = diameter of sphere, L = length of the rod and R = root-mean-square of the distance between ends of the random coil.

In all cases the function $P(\theta)$ tends towards unity as θ tends towards zero, there being no intraparticle interference in the direction of the incident beam.

It is sometimes more convenient to express $P(\theta)$ as a series expansion in θ . Equation 53 may thus be written as (ref. 59)

$$\frac{1}{P(\theta)} = 1 + \frac{n^2}{9} + \frac{7n^4}{2025} + \dots \quad (55)$$

where $n = (2\pi L/\lambda) \sin \theta/2$ and equation (54) as (ref. 59):

$$\frac{1}{P(\theta)} = 1 + \frac{n}{3} + n^2/36 + \dots \quad (56)$$

where $n = 8/3\pi^2 (R/\lambda)^2 \sin^2 \theta/2$.

In general, two methods have been used for the treatment of light scattering data. In the dissymmetry method the intensity of the scattered light is measured at two angles which are supplementary. The dissymmetry thus found may be used to calculate the particle size for any particle shape assumed, and the correction $P(\theta)$ which is to be applied to obtain the true molecular weight. Alternatively the intensity is measured at various angles and Kc/R_θ is plotted against concentration. The slope determines the second virial coefficient, and the intercept with the Kc/R_θ axis gives $M/P(\theta)$ which yields the reciprocal of the molecular weight on extrapolation to zero angle. Both extrapolations may conveniently be carried out in a

single graph by the Zimm method (67). Here Kc/R_θ is plotted against $\sin^2 \theta/2 + kc$, where k is an arbitrary constant chosen to spread out the data. Thus treated, the data form a grid in which extrapolated points are joined into two lines that should meet at the same intercept of Kc/R_0 at zero concentration. The reciprocal of the intercept is equal to the molecular weight. The value of B is obtained from the slope of the zero angle line, while the dimension can be calculated from the ratio of the slope of the zero angle line to the intercept, viz.

$$(\bar{R}^2)^{\frac{1}{2}} = \frac{9 \lambda'^2 (\text{limiting slope})}{8 \pi^2 (Kc/R_\theta)_{c=0}} \quad (57)$$

where λ' is the wavelength of the incident light in the solution. The molecular dimension may also be expressed in terms of the so-called radius of gyration, or root-mean-square radius from the centre of mass of the particle. It is related to the characteristic dimension by the expressions

$$\text{sphere:} \quad (\bar{R}_g^2) = 3 D^2/20, \quad (58)$$

$$\text{rod:} \quad (\bar{R}_g^2) = L^2/12, \quad (59)$$

$$\text{coil:} \quad (\bar{R}_g^2) = R^2/6. \quad (60)$$

Information about the shape of the particle may in principle be obtained by the comparison of the observed and calculated angular scattering functions.

In the case of a polydisperse system the light scattering method gives a weight-average molecular weight and a z -average molecular dimension; i.e. the dimension of a particle having a molecular weight, M_z . The form of the $1/P(\theta)$ function depends not only on the molecular size but also on the polydispersity of the sample (67). For the special case where the distribution corresponds to $M_z:M_w:M_n::3:2:1$ the plot of $1/P(\theta)$ against $\sin^2 \theta/2$ is linear and there are no difficulties in interpretation. When the molecular size is sufficiently large and the system polydisperse, the precise determination of the angular distribution of the scattered light can give M_n , M_z , M_w and the corresponding average dimensions (68).

Experimental methods and results

The measurements were made with an instrument constructed and calibrated by Dr. A. Bengtsson of this Institute. Details will be published elsewhere. The design is similar in many respects to others previously described (cf. e.g. ref. 67). It was operated on the "null" principle, i.e., the scattered light observed by a photomultiplier detector was balanced by means of an electronic bridge circuit against a portion of the incident beam which was diverted by a mirror system to a photoelectric tube. An electronic voltmeter was used to observe the balance of the opposed and amplified 100 cycle signals from the photomultiplier detector and the incident light monitor. The light source was a Philips Philora H 500 medium-pressure mercury lamp. The 5460 and 4358 Å lines were isolated by means of Zeiss filter combinations. Unpolarised incident light was used throughout.

In carrying out light scattering measurements it is important that the intensity of the light scattered from the solutions under study be directly compared with

that of a working standard material which has itself been calibrated in absolute terms. The absolute calibration was based upon reflectivity measurements of a MgO surface. Such a surface does not act as an ideal diffusor; there being always at least a small fraction of the light which undergoes spectral reflection, and a small correction was applied to take care of the non-ideality of the diffusor. The reflectivity of the diffusor was 0.97 at a wave length of 4358 Å. This absolute calibration was checked by measuring the excess turbidity of a 0.5 per cent solution of Dow Styron in toluene. The value obtained was $3.50 \times 10^{-3} \text{ cm}^{-1}$ at 4358 Å as compared with $3.51 \times 10^{-3} \text{ cm}^{-1}$ reported by Brice *et al.* (69). A neutral filter (optical density about 10^{-5}) placed in the primary beam and measured at zero angle served as a working standard.

Because of the strong dependence of the intensity of scattering on the volume of the particles, it is extremely important in light scattering measurements to eliminate dust and other foreign impurities which scatter a considerable amount of light even when present in relatively small concentrations. As a result of the great dissymmetry of the radiation envelope of dust particles, even greater care is required when angular dependence measurements are to be made. It has long been known that the optical clarification of aqueous solutions poses special problems and in this respect great difficulties were encountered in the present investigation. In initial experiments stock solutions were first cleaned by centrifuging for one hour at 30,000 r.p.m. in the large rotor of the Spinco preparative ultracentrifuge. The amount of material centrifuged out, as a gel-like deposit on the bottom of the cell, was in all cases very small and no change could be detected in the concentration of the solution. The solutions were carefully syphoned from the centrifuge tubes and then filtered through a Jena-G 5 filter directly into the light scattering cell. Even after this treatment abnormally high dissymmetries ($z_{45} = 2 - 4$) were observed for all fractions. Careful visual examination of the low angle scattering in green light with the aid of a lens revealed the presence of a very small amount of motes. This, however, could not account for the high dissymmetries observed.

Working on the hypothesis that the difficulties were due to undispersed particles of very high molecular weight, a number of experiments were carried out in an attempt to remove them.

1. Aqueous solutions were centrifuged at 30,000 r.p.m. for thirty-six hours. The middle portion of the solution in each tube was removed by careful syphoning and its dissymmetry measured.
2. The polymer was dissolved in 0.5*N* sodium hydroxide and the dissymmetry was measured. Higher concentrations of sodium hydroxide could not be used since the solutions thus obtained were highly turbid.
3. If aggregation due to the formation of random hydrogen bonds is present in polymer solutions, it should be dispersed by the addition of a hydrogen-bond breaking agent such as urea. This has been demonstrated by Alexander and Stacey (71) in the case of polyvinyl alcohol. In the present case such a type of aggregation seemed likely to occur, in view of the many hydroxyl groups in the polymer. The light scattering was measured in 3 and 6 molar urea solutions.
4. Solutions were heated to a temperature of 50°C for several hours and the dissymmetry was measured at this temperature. It was expected that the increased thermal motion might be sufficient to break up any aggregates present. Higher temperatures could not be used since, as pointed out before, gelation or flocculation would result.
5. Scattering measurements were made on solutions at a temperature of about 5°C. Prior to the measurements the solutions had been maintained at that temperature for several hours.

Since the solutions exhibit a negative temperature coefficient of viscosity, aggregates due to partial substitution of the cellulose chains might become molecularly dispersed at these low temperatures.

6. Dissymmetries were measured on solutions before and after freezing (see page 526).

7. Solutions were exposed to ultrasonic vibrations at a frequency of 600 Kc for about thirty minutes. Higher frequencies were not used because of the danger of degradation.

8. In the fractionation studies it had been found that large amounts of acetone could be added to the aqueous solutions of the polymer without causing any appreciable precipitation. Nevertheless, it was felt that aggregated material would perhaps be precipitated under such conditions. To one volume of aqueous polymer solution three volumes of acetone were added. After allowing the solution to stand for twenty-four hours it was centrifuged for one hour at 30,000 r.p.m. The clear solution thus obtained was poured into excess acetone in order to effect quantitative coagulation of the polymer which then dried by methods previously described. The polymer was then redissolved in water and the scattering was measured.

In all of the above experiments high dissymmetries were still obtained regardless of the fraction used.

It was finally found that there was a considerable decrease in dissymmetry after filtration through membrane filters (Membranfilter Gesellschaft, Göttingen, Germany). These filters are supplied in four grades; large, medium, fine and ultrafine having pore sizes of 0.75–3.0, 0.5–0.75, 0.2–0.5 and <0.2-microns respectively. Only the large size could be used since the others became completely clogged. Even for the large size concentrated solutions ($c > 0.3$ per cent) could not be filtered for the same reason.

The solutions were first cleaned by centrifugation as described above and then filtered under 1–2 kg/cm nitrogen pressure through the membrane filter clamped in a Seitz holder. Provided the solutions were sufficiently dilute no change in concentration could be detected but in all cases concentration determinations were made on filtered solutions. Dissymmetry-free water ($z_{45} = 1$) used for dilutions was obtained by filtering laboratory distilled water through large or medium size filters. Filtrations were made directly into the light scattering cell, a new filter being used for each solution. By the use of these membrane filters fewer precautions were needed in cleaning transfer pipettes and dilution flasks since dust left behind after the usual cleaning procedures could be removed by filtration.

The green line of mercury (5460 Å) was used in all the measurements after it had been established that the blue line (4358 Å) gave the same value of Kc/R_θ , showing thereby that fluorescence was negligible.

Scattering measurements were made on the solvent and a series of each sample prepared by volume dilution of a clarified stock solution, each solution being filtered individually. Measurements were only made when careful visual examination at low angles did not reveal any notes.

The dissymmetry of scattered light was rather large in most cases. Under such circumstances the extrapolation method is to be preferred since it is independent of assumptions of particle shape, makes use of more data, and yields more precise values of the molecular size and weight. For the angular intensity measurements a cylindrical cell having flat entrance and exit windows for the primary beam was used. Intensities measured at 7–8 angles covering the range from 40 to 135° were corrected for the following effects. (a) As unpolarised incident light was used, the scattered light contained a horizontal component proportional to $\cos^2 \theta$. Intensities

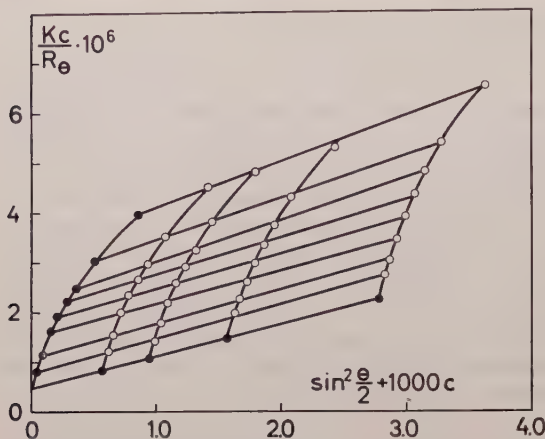


Fig. 18. Zimm plot for ethyl hydroxyethyl cellulose fraction M7 in water. The solutions were cleaned by high speed centrifugation followed by filtration through an ultrafine glass filter. The intercept $(Kc/R_θ)_{c \rightarrow 0}$ corresponds to a molecular weight of 2.08×10^6 .

were therefore normalised by multiplication with the factor $(1 + \cos^2 \theta)^{-1}$. (b) The volume of the scattering solution "viewed" by the receiver varies with the angle. This may be approximately corrected by multiplying the measured intensity by $\sin \theta$. (c) When the scattered light is incompletely polarised there may be a small contribution to the measured intensity arising from fluctuations in orientation of the solute. The application of this correction requires a knowledge of the depolarization $\rho = H_u/V_u$, where H_u and V_u denote the intensities of the horizontal and vertical components of the scattered light when the incident light is unpolarised. The depolarization was measured by means of a polaroid placed in the scattered beam in the 90° position. The average depolarization ρ for all fractions was found to be 0.02 and the molecular weight was multiplied by the Cabannes factor $(6-7\rho)/(6+6\rho)$. (d) The scattered light undergoes refraction at the cell wall. The correction for this effect was accomplished in the manner indicated by Hermans and Levinson (70) who have shown that the correction factor is proportional to the square of the refractive index of the solution, irrespective of the shape of the scattering cell, provided the receiver does not see past the edges of the beam. The geometrical arrangement of the apparatus was such that this requirement was met. (e) Intensities of the solvent were subtracted from those of the solution.

Reciprocal intensity plots were made for all fractions. Molecular weights were computed from intercepts of the bilinear extrapolation on the ordinate and molecular sizes were calculated according to equation 57. Fig. 18 shows a typical Zimm plot obtained when the solutions were filtered through glass filters. It indicates the presence of very large particles (weight average M_w , 2.08×10^6) but it is clear from the curvature in the angular scattering plots that the mixture is very polydisperse. The result obtained when a portion of the same solution was filtered under pressure through membrane filters is shown in Fig. 19. The marked change in the shape of the reciprocal scattering envelope and the considerable decrease in molecular weight ($M_w = 0.4 \times 10^6$) indicates that some of the large particulate matter had been removed by the membrane filtration. In fact, the linearity of the angular scattering curves leads one to believe that the solutions are free of all foreign matter. This plot is typical of that obtained for the other three fractions. In all cases the dissymmetry showed a very slight linear increase with decrease in concentration.

The results obtained are collected in Table 15.

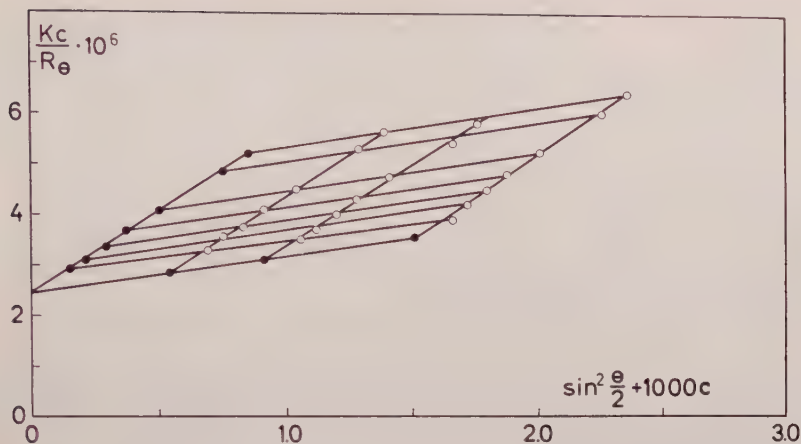


Fig. 19. Zimm plot for ethyl hydroxyethyl cellulose fraction M7 in water. The solutions were cleaned by high speed centrifugation followed by filtration through cellulose membranes. The intercept $(Kc/R_\theta)_{c \rightarrow 0}$ corresponds to a molecular weight of 4.0×10^5 .

Table 15. Molecular weights and dimensions of ethyl hydroxyethyl cellulose in water at 20°C as determined by light scattering.

Fraction No.	$(z_{45})_{c \rightarrow 0}$	$B \times 10^4$ mol. cm ³ g ⁻²	R_z [Å]	$M_w \times 10^{-3}$ By Zimm method	$M_w \times 10^{-3}$ from $(z_{45})_{c \rightarrow 0}^{(1)}$
12	2.0	1.5	1870	570	530
7	1.80	3.6	1630	460	410
5	1.70	1.7	1370	250	250
3	1.45	5.3	1270	160	170

¹ Assuming coil model.

PART 2

General discussion

(1) Molecular weights and polydispersity of the fractions

In Table 16 the molecular weights obtained by the different methods and the corresponding degrees of polymerization are assembled for purposes of ready comparison. It is immediately evident that the values from light scattering are about twice as great as those from sedimentation-diffusion and sedimentation equilibrium. The results from light scattering and sedimentation equilibrium should be the same since both methods give a weight average. There are three possible explanations for the discrepancy. (a) The light scattering apparatus is faulty and leads to a systematic error in the results. This possibility may be dismissed in view of the fact that the correct molecular weight was obtained for an International Polystyrene sample (72). (b) As a single stage fractionation was used, the samples may be very broad

Table 16. Molecular weights, degrees of polymerization and

Fraction No.	$M_n \times 10^{-3}$	$M_{ww} \times 10^{-3}$	$(M_w)_{\text{sed}} \times 10^{-3}$	$M_z \times 10^{-3}$ (¹)	$(M_w)_{\text{L.S.}} \times 10^{-3}$
M12	176	310	270	480	570
M7	128	200	200	310	400
M5	77.6	140	140	200	250
M3	52(¹)	92	90	160	160

(¹) deduced from relation between $[\eta]$ and $\bar{M}$.

(²) calculated from M_n and $(M_w)_{\text{sed}}$; assuming Kraemer-Lansing distribution.

and may have a distribution showing a long high molecular weight tail. (c) Each fraction contains a wide spectrum of high molecular aggregates. Although the greater part of these are removed by the method of cleaning the solutions, a very small portion persists and affects the light scattering results.

Before discussing these two latter possibilities we consider briefly the question of whether the light scattering data has been correctly interpreted.

Polydisperse systems of sufficiently high molecular weight may exhibit curvature of the reciprocal scattering envelopes (67). According to Benoit (68) two different molecular weight averages may then be obtained as follows:

$$\bar{M}_w = 1/(Kc/R_0)_{c=0},$$

$$\bar{M}_n = 1/(2 \times \text{intercept of asymptote}).$$

Thus it may be that in the present cases the measurements may have been made on the asymptote and a possible curvature at lower angles has been missed. If this is true then the molecular weights obtained are in reality twice the number-average molecular weight. Comparison with the osmotic pressure data reveals, however, that such an interpretation is impossible. This is further shown by the fact that the M_w values calculated by the Zimm and dissymmetry methods are in good agreement.

We consider next the question of the breadth of the distributions implied by the light scattering and osmotic molecular weights.

Perhaps the simplest means of expressing molecular heterogeneity is to compare the different average molecular weights $\bar{M}_n$, $\bar{M}_w$, $\bar{M}_z$. As pointed out before, in a monodisperse system all the averages will have the same value and from a qualitative standpoint it may be said that the greater the spread in the average molecular weights, the greater is the polydispersity. The unique evaluation of molecular size distributions in polymer systems is a very complicated problem. A method which has generally been used is to fit an assumed parametric function to the observed average molecular weights (cf. e.g. 38, 73). Of the several model distributions available it is convenient to choose the single-peaked Lansing-Kraemer logarithmic exponential function (74). It makes no difference if this does not really represent the actual distribution since it is only being used here for characterizing the polydispersity. The weight distribution is then defined as

$$f(M) dM = dw = \frac{w}{\bar{M}_n \beta \sqrt{\pi}} \exp \left(-\frac{1}{\beta} \ln M/M_0 \right)^2 dM \quad (61)$$

dispersity for fractions of ethyl hydroxyethyl cellulose.

$x_v \times 10^{-3}$	Z_n	Z_{ww}	$(Z_w)_{\text{sed}}$	$(Z_w)_{\text{L.S.}}$	$(M_w)_{\text{sed}}/M_n$
280	840	1480	1300	2900	1.5
190	610	960	940	2000	1.5
133	370	670	660	1200	1.8
86	250 ⁽¹⁾	430	430 ⁽¹⁾	800	1.7

where dw is the weight of polymer having a molecular weight between M and $M + dM$; w is the total weight of polymer (usually normalized to one), M_0 is the molecular weight at the maximum of the weight distribution curve, $\bar{M}_n$ is the number average molecular weight and β is a non-uniformity coefficient, equal to zero for a monodisperse sample. Equation (61) may also be expressed in integral form by plotting the function $F(M)$ as a function of M , where

$$F(M) = \int_0^M f(M) dM.$$

For polydisperse systems with the above distribution the following relationships are valid

$$\bar{M}_z/\bar{M}_w = \bar{M}_w/\bar{M}_n = e^{0.5\beta^2} \quad (62)$$

$$M_p/\bar{M}_w = e^{1.25\beta^2} \quad (63)$$

$$M_0 = M_p e^{0.5\beta^2} \quad (64)$$

Typical distribution curves constructed on the basis of the above relations using $\bar{M}_n$ from osmotic pressure and $\bar{M}_w$ from light scattering are shown in Fig. 20. For the sample in question (M5) the following characteristic constants were found $M_0 = 43,000$, $\beta = 1.5$. About 15 per cent of the material has a degree of polymerization greater than 2500. From our knowledge of the molecular weight of cellulose itself and taking into consideration the degradation which is known to occur during the preparation of this derivative, such a result seems highly unlikely. This is even more apparent when it is realised that this is one of the low molecular weight fractions.

Independent support for the opinion that the fractions do not possess the broad distribution indicated by the light scattering results was derived from two sources, namely from an analysis of the sedimentation velocity diagrams and by a comparison of polydispersity indices derived from sedimentation equilibrium and osmotic pressure on the one hand and the diffusion measurements on the other.

Several methods are available for the sedimentation analysis of polydisperse macromolecular systems (38, 75). The method used in the present case was developed by Eriksson (76) and successfully applied to a study of polydispersity in polymethylacrylate. Very briefly, the method is as follows. The integral distribution of sedimentation constants is determined at various points on the sedimentation curve.

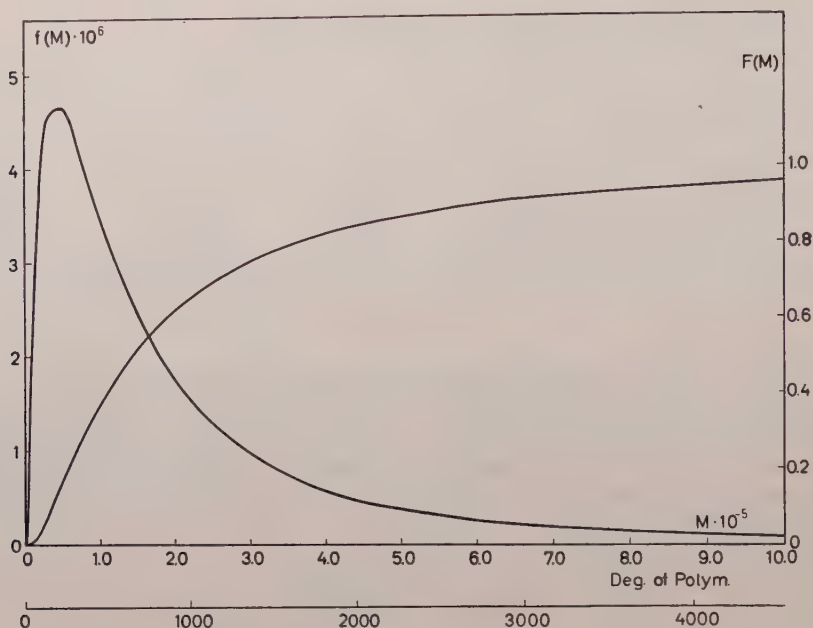


Fig. 20. Differential and integral distribution curves as calculated from the osmotic and light scattering molecular weights for fraction M5.

This apparent distribution is then extrapolated to infinite time and infinite dilution in order to eliminate the effects of diffusion and concentration. Fig. 21 shows the distribution of sedimentation constants for the fraction considered above. On the basis of the results from sedimentation-diffusion and sedimentation-equilibrium the scale on the abscissa corresponds to a molecular weight range of 90,000 to 300,000. Thus all the molecules in the sample have a degree of polymerization less than 1500.

Gralén (37) has shown that it is possible to calculate the Lansing-Kraemer non-uniformity coefficient β from diffusion measurements.

Assuming a logarithmic distribution of diffusion constants, viz.,

$$dc = K_D e^{-y^2} dD \quad (65)$$

where $y = (1/\gamma) \log (D/D_1)$, the diffusion constants as derived by the area and moment methods are related by the equation

$$D_A/D_1 = D_m/D_A = e^{3\gamma^2/8}. \quad (66)$$

Here K_D is a constant, γ is the distribution coefficient for the diffusion constants and D_1 is the diffusion constant at the maximum point of the distribution curve. If

$$M = \frac{\text{const}}{D_a} \quad \text{and} \quad M = \text{const } s^b$$

$$\text{then} \quad (a-1)(b-1) = 1 \quad (67)$$

$$\text{and} \quad \beta = \gamma a. \quad (68)$$

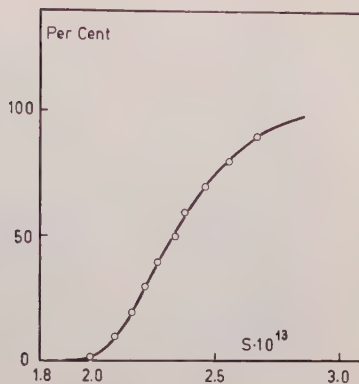


Fig. 21. Percentage of material with sedimentation constants less than S (fraction M5).

In Table 17 it is seen that the values of β calculated from light scattering-osmotic pressure are considerably higher than those derived from sedimentation equilibrium-osmotic pressure and diffusion. The values obtained from the two latter methods are in good agreement.

We turn now to the question of aggregation. Over the concentration range (0–0.3 per cent) at which the different measurements were normally made there was no evidence of a second component in the usual sedimentation diagrams; and observations during the acceleration period did not reveal any fast sedimenting molecular species. With the aid of a Schlieren optical system some observations made on 2 per cent solutions (not cleaned by preparative centrifugation) showed a small peak which sedimented within five minutes after the centrifuge was turned on. During the first 15–20 minutes of the acceleration period there was a considerable irregularity of the base lines which, however, later disappeared. Solutions which had been cleaned in the Spinco preparative ultracentrifuge did not show these effects.

From the preceding discussion the conclusion may be drawn that all the samples contain a small amount of aggregated material. A portion of this is removed when the solutions are cleaned in the Spinco preparative centrifuge. The amount which remains behind is too small to be detected in sedimentation velocity diagrams, if not centrifuged out already during the acceleration period. Thus molecular weights calculated from sedimentation data are not affected by the presence of such material.

Table 17. Values of the the Lansing-Kraemer non-uniformity coefficient as derived from the different experimental methods used.

Fraction No.	β		
	Light-scattering + osmotic pressure	Sedimentation equilibrium + osmotic pressure	Diffusion
M12	1.5	1.0	1.3
M7	1.5	0.94	1.08
M5	1.5	1.08	1.08
M3	1.5	1.07	1.08

The discrepancy between the molecular weights obtained from sedimentation-diffusion and sedimentation-equilibrium on the one hand and light scattering on the other, is due to a small amount of aggregated material present in the light scattering solutions in spite of the methods of clarification.

It is obvious that on a number basis the amount of aggregated material present in the solutions is negligible and hence would have no effect on the number average molecular weight. Several workers (cf. e.g. refs. 71, 77) have shown that aggregation has little or no effect upon viscosity even when present in a considerable degree. The same conclusion might be drawn in the present case since the same intrinsic viscosity was found for solutions cleaned by filtration through coarse glass filters (Jena G3) and by high speed centrifugation.

Finally, we consider briefly the question of the origin of the aggregates. It is clear from the various attempts at dispersion that extremely strong chemical valence forces are involved. As such forces are present in cellulose itself it seems most probable that the aggregation is due to the uneven distribution of substituents along the cellulose chains. An analogous situation appears to exist in the case of cellulose xanthate (78) and sodium carboxymethyl cellulose (79) and may be interpreted, following Höppler (81) and Ott (80), in terms of the peculiarities of the etherification reaction of cellulose. In the preparation of alkali cellulose and its subsequent conversion to ethyl hydroxyethyl cellulose a uniform distribution of substituents along the cellulose chain would not be expected, since accessible regions in the amorphous portions of the cellulose and on the periphery of the crystalline regions would react more rapidly than the interior of the highly crystalline portion. Upon dispersing the ethyl hydroxyethyl cellulose in water, while most of it dissolves molecularly there still remains a small percentage of molecules which are held together by the crystalline remnants from the original cellulose. That this view is essentially correct is supported by Fig. 22 which shows a number of photomicrographs of the gel-like deposit isolated from 2 per cent solutions of ethyl hydroxyethyl cellulose by high speed centrifugation. The pictures were obtained with the aid of phase contrast microscopy. In view of what has been said above, there is every reason to believe that the particles seen here merely represent the upper range of a wide spectrum of large particulate matter. The pictures reveal a number of interesting factors in connection with the preparation of alkali cellulose and the morphology of cellulose fibers. Further discussion of the matter is, however, beyond the scope of the present work.

This investigation therefore emphasises the fact, perhaps well known but mostly neglected, that the characterization of high molecular materials and cellulose derivatives in particular, solely on the basis of light scattering and viscosity measurements is not only unwise but decidedly hazardous.

As pointed out before, the weight average molecular weights from sedimentation equilibrium measurements are in good agreement with the weight-weight average from sedimentation-diffusion. It is known that $M_n < M_{ww} < M_w$, and for certain special theoretical distributions Jullander (38) and Singer (82) have shown that M_{ww} should be closer to the number than to the weight average. However, since in actual practice M_{ww} is often found to lie rather close to M_w (38, 83), the agreement obtained here is not unusual, and it seems justified to take the M_{ww} values as a very close approximation to M_w .

Values of M_w/M_n , used as measure of polydispersity, are given in Table 16. Values of M_z calculated on the assumption that the fractions have a distribution

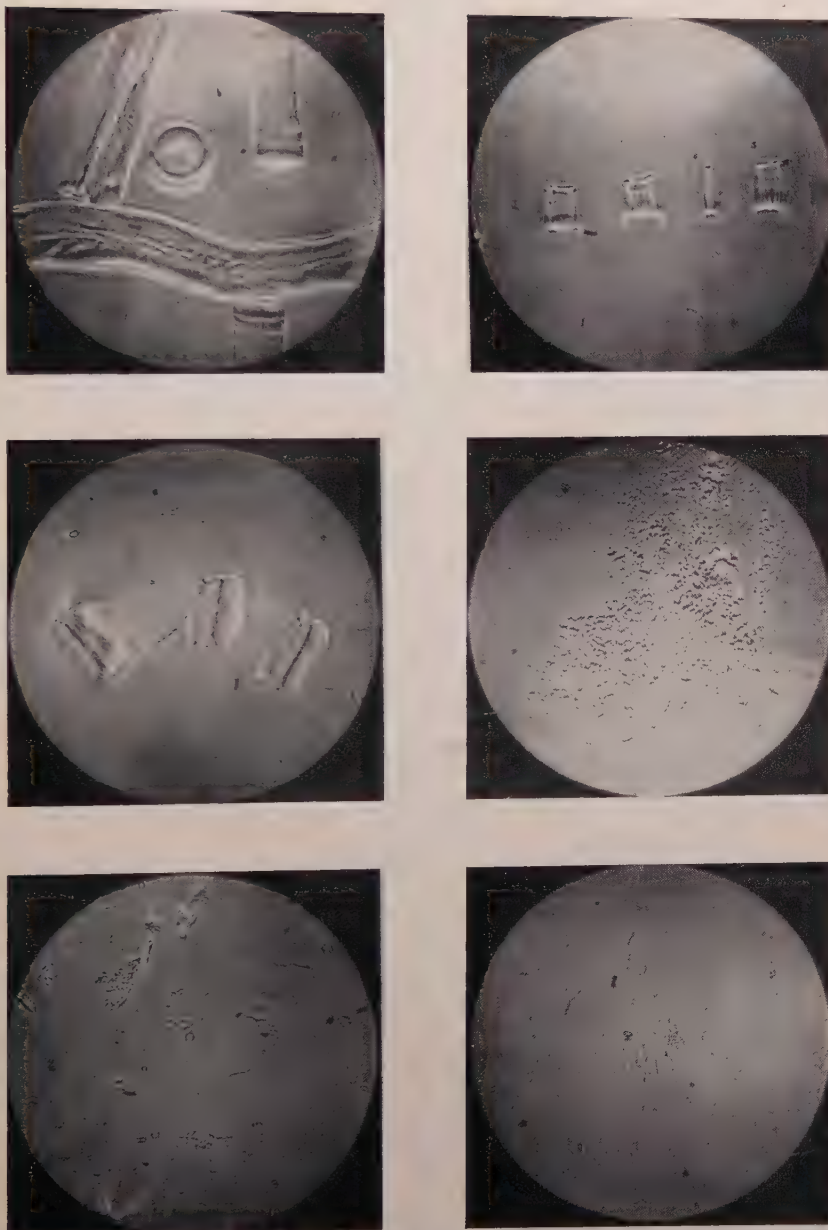


Fig. 22. Photomicrographs of the gel fraction isolated from 2 per cent solutions of ethyl hydroxyethyl cellulose. Fiber fragments of various sizes and state of swelling are seen.
(Magnification $320\times$.)

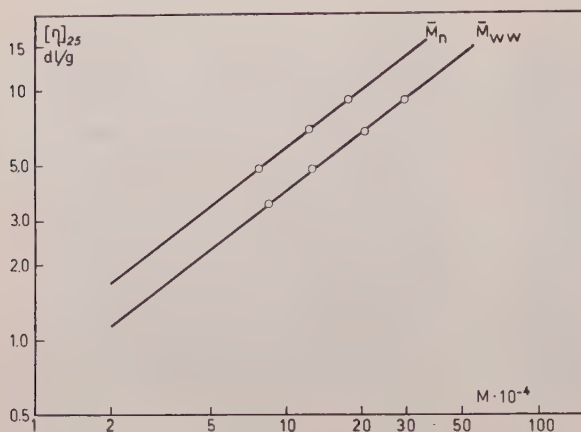


Fig. 23. Double logarithmic plots showing the dependence of intrinsic viscosity at 25°C on the number-average (M_n) and weight-weight (M_{ww}) average molecular weights for ethyl hydroxyethyl cellulose in water.

of the Lansing-Kraemer form are also given in the same Table. It is evident that all the fractions investigated are rather polydisperse. This, however, is precisely what one expects since only a single stage fractionation was used. It is expected that sharper fractions would have been obtained by refractionation but the difficult and time consuming nature of the method made such a course impractical. Furthermore, it is doubtful whether refractionation by this method would provide the sharpness necessary for more precise determinations of the molecular parameters (see also page 522).

(2) Molecular weight dependence of $[\eta]$, s and D

According to Staudinger (84), in solutions of threadlike molecules the intrinsic viscosity is proportional to the molecular weight. More recent investigations have, however, shown that this is a special case of the more general relation

$$[\eta] = KM^a$$

where K is a constant independent of molecular weight. Several theoretical developments, to be discussed shortly, indicate that the exponent a may have values lying between the extremes of 0 and 2 depending upon the flexibility of the polymer chains but not on the molecular weight. Three special cases are worthy of note. When a approaches the limiting value of 2 it implies that the molecules have a rod-like configuration. For free draining random coils $a = 1$, and $a = 0.5$ corresponds to a compact solvent immobilizing coil.

Fig. 23 shows a double logarithmic plot of $[\eta]_{25}$ against M_n and M_{ww} . The relation between intrinsic viscosity and molecular weight can be expressed by the equation

$$[\eta]_{25} = 3.7 \times 10^{-4} \bar{M}_{ww}^{0.8}. \quad (69)$$

Based on number average values the relation is given by

$$[\eta]_{25} = 4.70 \times 10^{-4} \bar{M}_n^{0.8}. \quad (70)$$

Relations (69) and (70) are reliable only for fractions having relatively narrow molecular weight distributions. In the comparison of samples with broad molecular

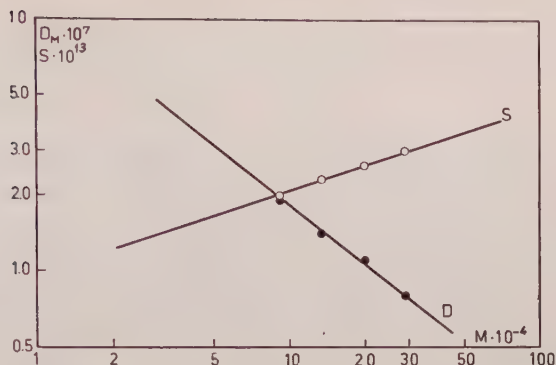


Fig. 24. Log-log plots of D_m and S against M_w for fractions of ethyl hydroxyethyl cellulose in water.

weight distributions it is more correct to use the relationship between the intrinsic viscosity $[\eta]$ and the viscosity average molecular weight (85) defined by

$$M_v = \left[\frac{\sum_i n_i M_i^{1+a}}{\sum_i n_i M_i} \right]^{1/a} \quad (71)$$

Flory (85) has indicated that when $0 < a < 1$,

$$M_n < M_v < M_w.$$

In order to calculate M_v it is necessary to make some assumption about the distribution. For the Lansing-Kraemer distribution (74) the following relation is valid

$$M_v = M_0 \exp \left\{ \frac{\beta^2}{2} (1 + a/2) \right\} \quad (72)$$

where the symbols have the previously mentioned significance. On the other hand the "most probable distribution" (86) is characterized by

$$\frac{M_v}{M_w} = \frac{[(1+a) \Gamma(1+a)]^{1/a}}{2} \quad (73)$$

where $\Gamma(1+a)$ is the gamma function of $(1+a)$.

Taking the value of the exponent a to be 0.8, values of M_v were calculated for the four fractions. Almost identical values were obtained from equations (72) and

Table 18. Dependence of the sedimentation and diffusion constants on molecular weight for different molecular models.

	Compact coil	Free draining coil
Diffusion constant	$D \sim M^{-1/2}$	$D \sim M^{-1}$
Sed. constant	$s \sim M^{-1/2}$	$s \sim M^0$

(73). The results are given in Table 16. The best straight line in the double logarithmic plot of M_v against $[\eta]$ is represented by the equation

$$[\eta]_{25} = 3.98 \times 10^{-4} M_v^{0.8}. \quad (74)$$

While it is possible that neither of these functions gives a really true description of the actual distribution in the fractions, the fact that the same relation is obtained when two different model distributions are used suggests that equation (74) is relatively insensitive to the distribution assumed and strongly supports its reliability.

Relations analogous to equations (69) and (70) exist between the molecular weight and the sedimentation and diffusion constants. Fig. 24 shows double logarithmic plots of s and D_M against molecular weight. The straight lines correspond to the relations

$$s = 3.1 \times 10^{-2} M^{0.36}, \quad (75)$$

$$D_M = 10^4 M^{-0.7}. \quad (76)$$

The exponent of the molecular weight has a characteristic value depending upon the shape of the molecules. Table 18 shows the dependence of the sedimentation and diffusion constants on molecular weight for the extreme cases of free draining and solvent-immobilizing coils (87).

From the exponent of the molecular weight in equations 69, 70, 75 and 76 the conclusion may be drawn that over the range of molecular weights studied, the molecule of ethyl hydroxyethyl cellulose has a form which lies between that of a free draining and solvent-immobilizing coil. Further information about molecular size and shape will be given in succeeding paragraphs.

(3) Relation of hydrodynamic behavior to molecular configuration

Several theories of linear polymers of random coil structure have been developed to account for the hydrodynamic interaction of the monomer units of the polymer and for the partial immobilization of solvent within the coil. These theories permit, at least in principle, the calculation of parameters which belong to well defined models and give a better picture of the molecular structure or behavior. Although the functional relationships arising from the several treatments are strictly applicable only to monodisperse systems, it is nevertheless of interest to consider the present results in terms of certain of these theories, in particular those of Kuhn and Kuhn, Debye and Bueche, Kirkwood and Risemann and Flory and Fox.

The theory of Kuhn and Kuhn (88) is based upon the model of a statistically coiled string of beads. From experiments on large scale wire models they deduced the intramolecular hydrodynamic interaction and arrived at the following formulae

$$[\eta] = \frac{N A_m b_m^2}{M_0} \frac{0.43 Z}{-1.6 + 2.3 \log_{10} (A_m/d_h) + (b_m Z/A_m)^{\frac{1}{2}}} \quad (77)$$

$$s = a_1 + b_1 \sqrt{Z} \quad (78)$$

$$D = (a_2 + b_2 \sqrt{Z}) Z^{-1} \quad (79)$$

where

$$a_1 = \frac{M_0}{N b_m} \frac{1 - V \varrho}{\eta_0} \left[-0.03 + 0.16 \log_{10} \left(\frac{A_m}{d_h} \right) \right] \quad (80)$$

$$b_1 = \frac{M_0}{N b_m} \frac{1 - V \varrho}{\eta_0} 0.136 \left(\frac{b_m}{A_m} \right)^{\frac{1}{2}} \quad (81)$$

$$a_2 = \frac{k T}{\eta_0 b_m} \left[-0.03 + 0.16 \log_{10} \left(\frac{A_m}{d_h} \right) \right] \quad (82)$$

$$b_2 = \frac{k T}{\eta_0 b_m} 0.136 \left(\frac{b_m}{A_m} \right)^{\frac{1}{2}} \quad (83)$$

for the intrinsic viscosity, sedimentation and diffusion constants.

where b_m is the hydrodynamic length of the monomer unit and d_h its thickness, A_m is the length of the statistical chain element, i.e., the length of the average number of monomer units after which a kink in the molecular chain occurs, Z is the degree of polymerization, k is Boltzmann's constant, N is Avogadro's number, M_0 is the molecular weight of the base unit and η_0 is the viscosity of the solvent.

The length A_m of the statistical chain element is determined by the two definitions (ref. 91)

$$L = Z b_m = K A_m, \quad \bar{R}^2 = K A_m^2$$

yielding

$$A_m = \bar{R}^2 / L \quad (84)$$

where L is the so-called hydrodynamic length, i.e., the fully extended length of the chain, as can be obtained without distortion of valence angles or atomic distances and $\bar{R}^2$ is the mean-square end-to-end distance of the chain.

For very high molecular weights the first two terms in the denominator of equation (77) can be neglected and $[\eta]$ becomes proportional to $Z^{\frac{1}{2}}$. For rigid chains of low molecular weight, for which $A_m = b_m Z$, $[\eta]$ becomes proportional to Z^2 .

In the treatment of Debye and Bueche (92) the coiled polymer molecule is replaced by a porous sphere of radius R_s uniformly filled with resisting points equal to the number of monomer units in the molecule. The degree of porosity of this sphere determines the hydrodynamic interaction. The flow of solvent through the aggregate of beads is treated by a modification of the Navier-Stokes equations leading to the results

$$[\eta] = \frac{4 \pi N}{3} \frac{R_s^3}{M} \Phi(\sigma) \quad (85)$$

$$[s] = \frac{s_0 \eta_0}{1 - V \varrho} = \frac{M}{6 \pi R_s N} \frac{1}{\psi(\sigma)} \quad (86)$$

$$D = \frac{k T}{6 \pi \eta_0 R_s} \frac{1}{\psi(\sigma)} \quad (87)$$

where $\Phi(\sigma)$ and $\psi(\sigma)$ are volume factors dependent on the "shielding ratio", σ . The latter is defined by the equations

$$\sigma = \frac{R_s}{K^{\frac{1}{2}}}, \quad K = \frac{(2R_s)^3}{36Zf}$$

where k is the permeability of the sphere, f is the frictional constant per monomer unit and Z is the degree of polymerization. R_s is related to the dimensions of the coil by the expression

$$2R_s = 1.054R$$

where R is the end-to-end distance.

If the swarm is very open, Staudinger's law is obtained as one limit. If, in the other limit, the swarm becomes impenetrable, the viscosity becomes proportional to $M^{\frac{1}{2}}$.

In its initial physical assumptions the Kirkwood-Riseman theory (93) is more rigorous. The model used is that of hampered flow through beads arranged as in a random coil. The hydrodynamic interaction between the different parts of the molecule is calculated directly on the basis of the classical hydrodynamic equations of motion. The relations obtained are

$$[\eta] = \frac{N\xi b^2 Z}{3600\eta_0 M_0} F(\lambda_0 Z^{\frac{1}{2}}) \quad (88)$$

where

$$\lambda_0 = \xi / (6\pi^3)^{\frac{1}{2}} \eta_0 b \quad (89)$$

and

$$F(x) = F(\lambda_0 Z^{\frac{1}{2}}) = 6/\pi^2 \sum_{k=1}^{\infty} \frac{1}{k^2} \frac{1}{1+x/k^{\frac{1}{2}}} \quad (90)$$

$$Z = M/M_0.$$

The independent molecular parameters are Z the degree of polymerization, b the effective bend length, i.e., the length of the statistically independent units of the chain, ξ the frictional coefficient of the single beads and η_0 the viscosity of the medium. From equations (88) to (90) it follows that as Z varies from 0 to ∞ the dependence of $[\eta]$ on M varies from M to $M^{\frac{1}{2}}$.

The frictional resistance associated with the translational motion of the flexible molecule is given by

$$f_T = \frac{Z\xi}{1 + \frac{8}{3}\lambda_0 Z^{\frac{1}{2}}} \quad (91)$$

whence the sedimentation and diffusion constants are obtained as

$$D = \frac{kT}{Z\xi} [1 + \frac{8}{3}\lambda_0 Z^{\frac{1}{2}}] \quad (92)$$

$$s = \frac{M_0}{N\xi} (1 - V\rho) [1 + \frac{8}{3}\lambda_0 Z^{\frac{1}{2}}]. \quad (93)$$

The Debye-Bueche and Kirkwood Riseman theories emphasize the degree of coil permeability as a function of molecular weight. In a more recent theory Flory and

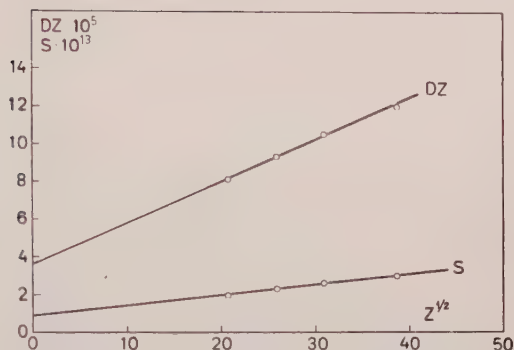


Fig. 25. Plots of DZ and s against $Z^{1/2}$ for ethyl hydroxyethylcellulose in water. D , s and Z are respectively the diffusion and sedimentation constants and the degree of polymerization.

Fox (94, 95) concentrate on the change of the coil dimensions with M . They postulate that the intrinsic viscosity of a polymer in solution should be proportional to the ratio of the volume occupied by the molecule to the molecular weight. As a measure of the volume of the molecule they use the cube of the root-mean-square end-to-end distance and they show that as the combined result of long range interactions between segments and of segment-solvent interaction, the molecular dimensions will be increased by a factor α . The intrinsic viscosity is thus given by the following relations

$$[\eta] = K M^{1/2} \alpha^3, \quad \alpha = (\bar{R}^2 / \bar{R}_0^2)^{1/2} \quad (94)$$

$$K = \Phi (\bar{R}_0^2 / M)^{3/2} \quad (95)$$

$$C_T = 2 \psi_1 C_M (1 - \theta / T) = (\alpha^5 - \alpha^3) / M^{1/2} \quad (96)$$

$$[\eta]^{1/2} / M^{1/2} = K^{1/2} + K^{1/2} C_T M / [\eta] \quad (97)$$

where K is a constant characteristic of the polymer and the temperature, $(\bar{R}_0^2)^{1/2}$ is the root-mean-square end-to-end distance in the absence of perturbation due to the solvent and long range interaction; Φ is a hydrodynamic constant (for sufficiently high molecular weights) which should be the same for all polymers irrespective of solvent, T is the absolute temperature, ψ_1 and C_M are thermodynamic constants, α is the ratio of the actual root-mean-square end-to-end distance to the unperturbed value, θ is the critical miscibility temperature for the polymer-solvent (of infinite molecular weight) system.

By an analogous argument Mandelkern and Flory (96) show that the frictional coefficient may be represented by the relation

$$f_0 / \eta_0 = P (\bar{R}^2)^{1/2} = P \left(\frac{\bar{R}_0^2}{M} \right)^{1/2} M^{1/2} \alpha \quad (98)$$

where P is a "universal" constant analogous to Φ of the viscosity theory and η_0 is the viscosity of the solvent. By combining equations (20), (94), (95) and (98) the following relation between the sedimentation constant and the intrinsic viscosity is obtained:

$$s_0 [\eta]^{1/2} / M^{1/2} = \Phi^{1/2} P^{-1} N^{-1} (1 - V_0) / \eta_0 \quad (99)$$

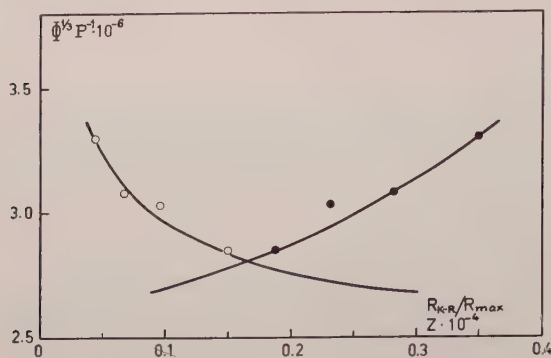


Fig. 26. Variation of $\Phi^{1/2} P^{-1}$ with extension ratio R_{K-R}/R_{max} (filled circles) and degree of polymerization (open circles).

The corresponding equation for the relation between the diffusion constant and the molecular weight is

$$D_0 \eta_0 (M[\eta])^{1/2} = k T P^{-1} \Phi^{1/2} \quad (100)$$

where k is the Boltzmann constant and T the absolute temperature. The theory predicts that for a series of homologous polymer fractions in a given solvent, the left hand member of equations 99 and 100 should be independent of M .

We turn now to an evaluation of the present experimental data in the light of the above theories. According to the theory of Kuhn and Kuhn the dependence of s and D on Z is given by equations 78 and 79. By introducing the experimentally observed values of a_1 , b_1 , a_2 , b_2 into equations (80) to (83) the parameters A_m and d_h can be determined since all the other parameters are known from the experimental conditions. In Fig. 25, s_0 and DZ are plotted against $Z^{1/2}$. From the slope and intercept of these lines and using the values $M_0 = 210$, $b_m = 5.15 \times 10^{-8}$ cm, $\bar{V} = 0.7$ it follows that

$$A_m = 120 \text{ \AA} \text{ and } d_h = 0.1 \text{ \AA}.$$

The sedimentation and diffusion data lead to exactly the same result. Knowing A_m and d_h the intrinsic viscosities were calculated for the four fractions. The results given in Table 19 are in all cases about 50 per cent lower than the experimentally observed values. The value of A_m seems to be of the right order of magnitude and corresponds on the average to about 23 glucose rests per link. The molecule is thus appreciably uncoiled. For cellulose acetate in acetone and methyl cellulose in water,

Table 19. Intrinsic viscosities and molecular dimensions of ethyl hydroxyethyl cellulose as calculated from the theories of Debye-Bueche (D-B), Kirkwood-Riseman (K-R) and Kuhn-Kuhn (K-K).

Fraction No.	$[\eta]_{20}$ obs. dl/g	$[\eta]_{20}$ D-B dl/g	$R_s(\text{\AA})$ D-B	$R(\text{\AA})$ D-B	$[\eta]_{20}$ K-K dl/g	$R(\text{\AA})$ K-K	$[\eta]_{20}$ K-R dl/g	$R(\text{\AA})$ K-R	R_{K-R}/R_{max}
12	9.4	3.86	396	750	4.36	960	10.8	1430	0.19
7	7.0	2.70	307	580	3.17	770	7.48	1140	0.23
5	5.0	1.94	244	460	2.24	640	5.55	960	0.28
3	3.7	1.10	176	330	1.73	514	3.80	770	0.35

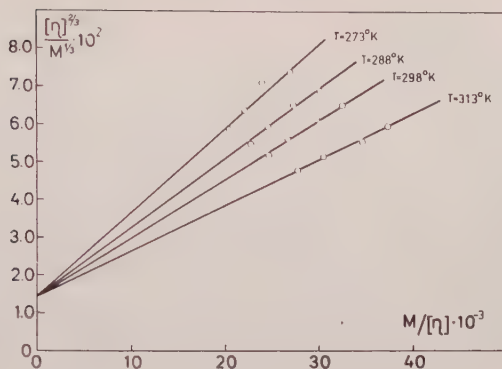


Fig. 27. Plots of $[\eta]^{3/2}/M^{1/2}$ versus $M/[\eta]$ for fractions of ethyl hydroxyethyl cellulose in water at various temperatures. The intercept corresponds to $K = 18 \times 10^{-4}$ (see equation (97)).

values of 110 Å and 50 Å respectively have been reported (88). On the other hand the value of d_h is somewhat unrealistic. For the two systems just mentioned the values are respectively 11 Å and 8 Å. This may indicate that the resistance of the coil to translational and rotational motion does not depend on the frictional resistance of the monomer in the manner suggested by the equations.

For the determination of R_s and σ in the Debye-Bueche theory any of the equations (85), (86) and (87) together with its logarithmic derivative

$$\frac{d \log [\eta]}{d \log M} = \varepsilon_1 = -1 + \frac{1}{2} \frac{d \log \phi}{d \log \sigma} + \left(3 - \frac{1}{2} \frac{d \log \phi}{d \log \sigma} \right) \frac{d \log 2 R_s}{d \log M} \quad (101)$$

$$\varepsilon = \frac{d \log s}{d \log M} = 1 - \varepsilon_2 = \left(1 - \frac{1}{2} \frac{d \log \psi}{d \log \sigma} \right) \left(1 - \frac{d \log 2 R_s}{d \log M} \right) \quad (102)$$

$$\frac{d \log D}{d \log M} = -\varepsilon_2 \quad (103)$$

may be used (91).

From the value of ε_1 and ε_2 as determined from the experimentally observed dependence of $[\eta]$ and s on M (see equations (69) and (75)) the values of the shielding ratio and volume factor were found by interpolation in tables given by the authors. The results are

$$[\eta] = K_\eta M^{\varepsilon_1}; \quad \varepsilon_1 = 0.8, \quad \sigma = 3.6; \quad \Phi(\sigma) = 0.74,$$

$$s = K_s M^{\varepsilon_2}; \quad \varepsilon_2 = 0.64; \quad \sigma = 3.4; \quad \psi(\sigma) = 0.65.$$

Values of R_s were then calculated by substitution in equation (86). Finally from a knowledge of R_s and $\Phi(\sigma)$ values of $[\eta]$ were calculated from equation (85). The results shown in Table 19 are about 50 per cent lower than the observed values.

The parameters ξ and λ_0 in the Kirkwood-Riseman theory were evaluated in accordance with equation 93 from the slope and intercept of the s against $Z^{\frac{1}{2}}$ plot. The effective bond length b was then calculated by substitution in equation (89). The values obtained are

$$b = 37 \text{ Å}; \quad \xi = 11.2 \times 10^{-10} \text{ g/sec.}; \quad \lambda_0 = 22.9 \times 10^{-3}.$$

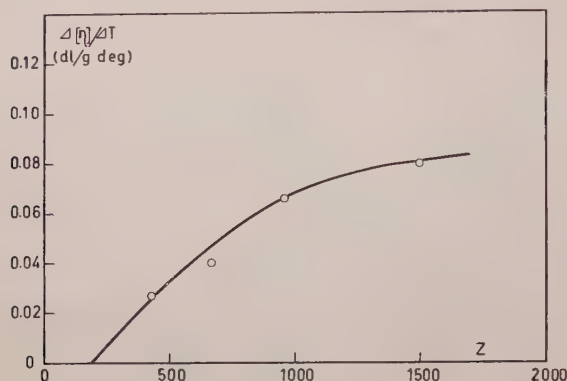


Fig. 28. Variation of temperature coefficient of intrinsic viscosity with degree of polymerization for ethyl hydroxyethyl cellulose in water.

For cellulose nitrate in acetone (97) and polystyrene in toluene (98) values of b of 35 Å and 8.6 Å respectively have been reported. The high value of b obtained here again indicates that the molecules are highly extended.

Intrinsic viscosities calculated from equation (88), using values of $F(\lambda_0 Z^{\frac{1}{2}})$ tabulated by Kirkwood and Riseman, are given in Table 19. For all fractions there is reasonable agreement with the observed values.

Molecular dimensions calculated from these three theories are given in Table 19. From the foregoing it is clear that the K - R values are to be considered the most reliable. It is to be expected that estimates made on the basis of various theories will differ, because of the different assumptions made. The fundamental relationships of the Debye-Bueche and Kirkwood-Riseman theories are essentially identical except for numerical factors (cf. e.g. ref. 99). Since in all the above calculations average molecular parameters have been used, the dimensions obtained are also average values.

According to equation (99) the quantity $s[\eta]^{\frac{1}{2}}/M^{\frac{1}{2}}$ evaluated for a series of polymer fractions should be constant independent of the nature of the polymer, the solvent and the temperature. The values given in Table 20 show a tendency to decrease with increasing molecular weight. Values of $\Phi^{\frac{1}{2}}P^{-1}$ calculated according to equations 99 and 100 also exhibit the same trend. This decrease is larger than can be accounted for either by the observed variations in the factor $(1 - V_0)/\eta_0 N$ (see Table 20), by the probable error of the measurements or by the polydispersity of the fractions. The effect of the latter would be to cause an increase in the absolute value of $\Phi^{\frac{1}{2}}P^{-1}$ for each fraction (100). For other linear polymer systems values of $\Phi^{\frac{1}{2}}P^{-1}$ range from 2.3 to 2.7×10^6 . Mandelkern *et al.* (101) have summarized the

Table 20. Values of $\Phi^{\frac{1}{2}}P^{-1}$ for ethyl hydroxyethyl cellulose in water.

Fraction No.	$\frac{s[\eta]^{\frac{1}{2}}}{M^{\frac{1}{2}}} \times 10^{17}$	$\frac{1 - V_0}{\eta_0 N} \times 10^{21}$	$(\Phi^{\frac{1}{2}}P^{-1})_{\text{sed}} \times 10^6$	$(\Phi^{\frac{1}{2}}P^{-1})_{\text{diff}} \times 10^6$
12	14.1	4.888	2.90	2.80
7	14.7	4.921	3.01	3.05
5	14.7	4.799	3.08	3.08
3	15.2	4.619	3.30	3.29

work of many authors and report an average of 2.5×10^6 . In Fig. 26, $\Phi^{\frac{1}{2}} P^{-1}$ is plotted against the degree of polymerization, Z . From the shape of the curve it appears that a limiting value of $2.5\text{--}2.7 \times 10^6$ would be reached at a value of Z somewhat greater than 2500, or a molecular weight of about 500,000.

The cause of this behavior probably lies in the high extension of the cellulose molecule at low and moderate degrees of polymerization. The Flory-Fox theory is only valid for those polymer systems in which the extension of the molecules is so low that their spatial distribution is spherically symmetric. There must therefore be a considerable degree of solvent immobilization. As pointed out by Flory (102), such a condition will only hold if the polymer molecules are irregularly coiled to an extent such that the root-mean-square end-to-end distance $(\bar{R}^2)^{\frac{1}{2}}$ does not exceed one-tenth of $(\bar{R}^2)_{\text{max}}^{\frac{1}{2}}$, the length of the molecule at full extension. For vinyl and similar polymers this is normally true down to molecular weights of $2\text{--}4 \times 10^4$. In Table 19 the values of the extension ratios $(\bar{R}^2)^{\frac{1}{2}}/(\bar{R}^2)_{\text{max}}^{\frac{1}{2}}$ are given. $(\bar{R}^2)_{\text{max}}^{\frac{1}{2}}$ is obtained as the product of the degree of polymerization and the length of the glucose residue (5.15 Å). The values of $(\bar{R}^2)^{\frac{1}{2}}$ are those calculated from the Kirkwood-Riseman theory. The extension ratios are rather high (see also Table 10) and the increase in $\Phi^{\frac{1}{2}} P^{-1}$ is paralleled by an increase in the extension ratio. Furthermore, as shown in Fig. 26, $\Phi^{\frac{1}{2}} P^{-1}$ shows a tendency to approach the accepted value at extension ratios less than 0.1.

A similar situation is found when the constant Φ is calculated from equations (94) and (95) using M_{wv} and $(\bar{R}^2)^{\frac{1}{2}}$ from the Kirkwood-Riseman theory. It is found to have values ranging from 0.74×10^{21} at low M to 0.96×10^{21} at high M in contrast to 2.1×10^{21} for Gaussian coil polymers. These results are quite in keeping with the previous conclusions as to the configuration of the ethyl hydroxyethyl cellulose molecule in solution. All the evidence indicates that, as with other cellulose derivatives (103), the molecules here are linear with a very open and almost free-draining configuration.

It is of interest to consider the effects of interactions between the glucose residues in the chain. For an unbranched chain the mean-square end-to-end distance is related to the degree of polymerization by the equation (refs. 104, 105)

$$b^2 Z = \bar{R}^2 = Z l^2 \left(\frac{1 + \cos \theta}{1 - \cos \theta} \right) \left(\frac{1 + \overline{\cos \varphi}}{1 - \overline{\cos \varphi}} \right) \quad (104)$$

where l is the bond length, equal to 5.15 Å, θ is the supplement of the valence angle—equal to 70° for cellulose and its derivatives, and $\overline{\cos \varphi}$ is the mean value of the cosine of the angle between successive bond planes. Using the value of b calculated from the Kirkwood-Riseman theory, $\overline{\cos \varphi}$ is found to be 0.924. Since $\overline{\cos \varphi}$ is zero if there is free rotation about the bonds it is evident that the chain is very greatly extended over the free rotation size. This effect is responsible for the lack of flexibility in cellulose and its derivatives. In the present case it is probable that the hindrance to rotation about the oxygen linking the glucose units is somewhat enhanced by the side groups.

Finally it is possible to relate the activation energy of viscous flow to the parameters α and K in the Flory-Fox viscosity theory by the equation (ref. 106)

$$\frac{d \ln [\eta]}{d(1/T)} = 3 \frac{d \ln \alpha}{d(1/T)} + \frac{d \ln K}{d(1/T)}. \quad (105)$$

It follows that the temperature coefficient of viscosity is due partly to the swelling of the molecules, $d \ln \alpha / d(1/T)$, and partly to internal modifications in the structure of the molecule ($d \ln K / d(1/T)$) caused, for example, by steric hindrance in the rotation of the segments.

The Flory theory cannot be used for the quantitative calculation of molecular dimensions in the range of molecular weights studied here; it does, however, allow certain qualitative conclusions to be drawn as to the relative importance of α and K in equation (105). According to equation (97), K may be ascertained from the intercept of a plot of $[\eta]^3/M^{\frac{1}{2}}$ against $M/[\eta]$. As seen in Fig. 27, a long and uncertain extrapolation is involved but within the limits of the experimental error K is independent of temperature. This is in sharp contrast to the behavior of other cellulose derivatives (107) and indicates that in the present system the change of viscosity with temperature is largely the result of swelling effects which undoubtedly arise from the increased molecular hydration at low temperatures.

Support for this point of view is also found in the variation of intrinsic viscosity with temperature (see Fig. 7). The decrease in viscosity with increase in temperature implies a corresponding change in solubility. This is, of course, contrary to normal solubility behavior and must mean that an addition compound is formed with water which is less stable at higher temperatures. Thus it is not ethyl hydroxyethyl cellulose itself which is soluble in water but rather its reaction product with water.

In Fig. 28 the temperature coefficient of viscosity is given as a function of the degree of polymerization, Z . The temperature coefficient vanishes at a degree of polymerization of about 200, indicating that at lower values the molecule is so highly extended that the swelling effects cease to be of importance. From this and the previously discussed behavior of the quotient $\Phi^{\frac{1}{2}}P^{-1}$, it is expected that the relations between the molecular weight and the various frictional constants, $[\eta]$, s and D should be valid for degrees of polymerization between 200 and 2500. Beyond these limits changes in the form of the molecule should be reflected in the value of the molecular weight exponent.

Summary

The non-ionic water soluble cellulose ether ethyl hydroxyethyl cellulose was fractionated by extraction with acetone-water solutions of varying acetone content. The method gave fractionation both with respect to degree of polymerization and chemical composition. The later effect was, however, confined to the species of lowest degree of polymerization. Of twelve fractions obtained four were selected for the characterization of the polymer.

Viscosity measurements were made at four temperatures in the range 0–40°C. The influence of surface tension and velocity gradient was investigated and found to be negligible. The solutions showed high intrinsic viscosities and high negative temperature coefficients of viscosity.

Number average molecular weights were determined from osmotic pressure measurements using a Fuoss-Mead osmometer with interferometric registration of height differences. Values obtained ranged from 50,000–180,000.

In ultracentrifugation studies the sedimentation curves showed a well defined fairly symmetrical peak. The sedimentation coefficients were extrapolated linearly

to infinite dilution in accordance with the relation

$$s = s_0 (1 + kc + k'c^2).$$

The values of the sedimentation constants lay in the range 2–3 Svedberg units.

Diffusion coefficients, measured by the free boundary technique with the aid of the Lamm scale method, showed a linear concentration dependence and were extrapolated to infinite dilution graphically. The diffusion constants thus obtained lay in the range $0.8\text{--}1.9 \times 10^{-7}$ cm²/sec.

Molecular weights computed from the Svedberg equation ranged from 90,000–300,000. Frictional coefficients, axial ratios and molecular dimensions were calculated from the sedimentation–diffusion data. The samples were characterized by high axial ratios and high frictional coefficients.

Sedimentation equilibrium measurements permitted the estimation of weight average molecular weights. The results showed good agreement with those from sedimentation–diffusion.

Angular dependence light scattering measurements gave molecular weights which were 80–100 per cent higher than those from sedimentation–diffusion and sedimentation equilibrium. The discrepancy is shown to originate in a small quantity of large particulate matter present in the solutions despite all efforts at optical clarification. The aggregated material is presumed to arise from the biological structure elements of the original cellulosic material.

Relations between molecular weight and intrinsic viscosity, sedimentation and diffusion constants are given.

The interpretation of the data in terms of current hydrodynamic theories leads to the conclusion that the molecules are hydrated linear chains coiled in an approximately free draining fashion.

Finally, the high temperature coefficient of viscosity is shown to be due to changes in the stability of the addition compound formed between polymer and solvent.

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Biologisch wirksame Oligo-Elemente in organischer Bindung

VON HANS V. EULER, HANS HASSELQUIST und BETH V. EULER

Unter den organisch gebundenen Oligo-Elementen sind es besonders die *Oligo-Metalle* (Oligo-Kationen), deren Studium wegen ihrer biochemischen und allgemein biologischen Bedeutung nunmehr — ein halbes Jahrhundert nach Erscheinen der ersten grundlegenden Untersuchungen von GABRIEL BERTRAND¹ — von mehreren Arbeitskreisen wieder aufgenommen worden ist. Es handelt sich bei diesen Oligo-Metallkomplexen meist um Covalenz-Verbindungen, z.T. auch um Einschluss-Verbindungen verschiedener Stabilität.

Unter den organisch gebundenen *Oligo-Metalloiden* hat in erster Linie das *Arsen* als Bestandteil hervorragend wichtiger Heilmittel — es sei nur an das Salvarsan erinnert — systematische Bearbeitung gefunden. Aber auch andere Metalloid-Anionen, z.B. der *Selenite* und *Selenate* und metallhaltige Anionen, z.B. der Vanadinsäure und Molybdänsäure, erwiesen sich, organisch gebunden, in minimalen Konzentrationen als biologisch wirksam. Auf einige derselben haben sich unsere Versuche konzentriert.

Biologische Wirkungen von Selen-Verbindungen

Zum Verständnis der noch wenig bekannten biochemischen Wirkungen von Selenderivaten sei einleitungsweise an einige chemische Tatsachen erinnert.

Selenwasserstoff ist, im Gegensatz zu Schwefelwasserstoff, eine mässig starke Säure (Diss. Konst. $K_1 = 2 \cdot 10^{-4}$, also grösser als die K der Essigsäure) und ist auch ein stärkeres Reduktionsmittel als H_2S .

Noch in 10^{-5} molarer Lösung (pH 5,5) erniedrigt SeH_2 bzw. SeH^- die Keimfähigkeit der Gerstensamen auf etwa $\frac{2}{3}$. Bemerkenswert ist, dass Selen den Schwefel im Cystein und in einigen anderen SH-Derivaten vertreten kann.

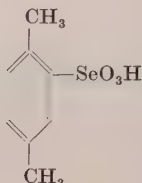
Natrium-Selenit, Na_2SeO_3 , ist im Gegensatz zu Natriumsulfit ein schwaches Reduktionsmittel, dagegen ein starkes Oxydationsmittel.³ Dichlorphenol-indophenol wird von Selenit weder in essigsaurer noch in alkalischer Lösung reduziert. Dagegen wird sowohl Ascorbinsäure als Triose-Redukton bei pH 6,5, Cystein auch in alkalischer Lösung, durch Na-Selenit unter Bildung von rotem Selen oxydiert. Alkali-Selenate sind starke Oxydationsmittel.

¹ Siehe u.a. G. BERTRAND, C. r. Acad. Sci. Paris 141, 1255, 1905. — G. BERTRAND et M. JAVILLIER, C. r. Acad. Sci. (Paris) 143, 900, 1906. Übersicht über die älteren Arbeiten von G. BERTRAND in Jubilé Scientifique de M. Gabriel Bertrand, Paris, Gauthier-Villars, 1938.

² Selen in Ackerböden und in Pflanzen: Siehe L. A. MAYNARD, Ann. Rev. Biochemie 10, 449, 1941.

³ Siehe Th. BERSIN, Erg. d. Enzymforsch. 4, 73, 1955.

In der Annahme, dass die Resorption der Selensäure bzw. selenigen Säure in der pflanzlichen und tierischen Zelle durch ihre Verbindung mit einem aromatischen Rest erleichtert wird, haben wir einige biologische Versuche mit p-Xylol-selenosäure angestellt, das zu diesem Zweck im Wesentlichen nach *Anschütz u. Mitarb.*¹ synthetisiert wurde.



2,3 ml p-Xylol und 2,0 ml Essigsäureanhydrid wurden auf 0° abgekühlt und tropfenweise mit 0,5 ml Selensäure unter Umschütteln versetzt, bis eine homogene Lösung entstand. Nach 5stündigem Stehen im Kälteschrank über Nacht und weiteren 5 Stunden bei Zimmertemperatur wurde die klar rote Lösung mit dem 4fachen Volumen Äther versetzt, wodurch ein amorpher Niederschlag entstand, der abfiltriert wurde. Nach Abdunsten des Äthers wurde der Rückstand mit Chloroform extrahiert. Der Extrakt hinterliess nach dem Einengen ein violettrotes Öl, das mit einigen Millilitern Essigsäureanhydrid versetzt und auf 0° abgekühlt beim Reiben kristallisierte. Das Reaktionsprodukt fiel in Form von hellrosa Nadeln aus, die mit kaltem Essigsäureanhydrid gewaschen, aus wenig Essigsäureanhydrid umkristallisiert wurde. Das sehr hygroskopische Produkt wird im Exsikkator über P₂O₅ und Natronkalk getrocknet. Die Kristalle schmelzen bei 95–96°, lösen sich leicht in Benzol und in Chloroform.

Keimungsversuche

Während die Keimfähigkeit von Gerstensamen, 3 g Samen in 100 ml Lösung, durch 1 mg Na-Selenit bis auf $\frac{1}{40}$ des normalen Wertes erniedrigt wird², hatte in analogen Versuchen 1 mg Na-Selenat nur eine Erniedrigung der Keimfähigkeit auf $\frac{1}{10}$ des normalen Wertes zur Folge.

Versuche mit unserer p-Xylol-selensäure ergaben, dass diese Substanz bei pH = 6,0 nach Vorbehandlung in Lösung von 10 mg in 100 ml angenähert die gleiche Keimungs-Erniedrigung (Mittel aus 3 Versuchen $\frac{1}{3-1/2}$ des normalen Wertes) hervorruft wie die Selensäure.

Versuche mit Yoshida-Ascites-Sarkom-Ratten

Die frische Ascites-Flüssigkeit wurde mit der physiolog. Kochsalzlösung der Selenit- oder Selenat-Dose 3,5 Stunden bei 20° aufbewahrt und dann bei 20° eingespritzt.

Die tumorhemmende Wirkung von Na-Selenit und von Na-Selenat wurde bereits in einer früheren Mitteilung von EULER, RESEGOTTI und HASSELQUIST (l.c.) erwähnt. Nach unseren späteren Versuchen erhöhten Dosen von 0,6 mg Selenit die Lebensdauer um einige Tage.

¹ R. ANSCHÜTZ, J. KALLEN u. K. RIEPENKRÖGER, Ber. Dtsch. chem. Ges. 52, 1860, 1919.

² Siehe hierzu EULER, L. RESEGOTTI u. H. HASSELQUIST, Zs. f. Naturforsch. 11b, 178, 1956. Ferner: BYERS, Ind. and Eng. Chem. News Ed. 12, 122, 1934.

Versuche mit 0,9 mg p-Xylol-selensäure zeigten keine tumorhemmende Wirkung dieser zu kleinen Dose; auch ihre Verdoppelung war ohne deutlichen Effekt. Wir beschränken uns deshalb auf die Mitteilung einer Versuchsreihe, bei welcher die Ratten an 2 aufeinanderfolgenden Tagen erhielten:

2mal 0,9 mg = 1,8 mg p-Xylolselensäure + 0,3 mg Na-Selenit.

Gewicht der Ratten 130–160 g. Die 5–8 Tage nach der Einimpfung des mit der Selendose gemischten Ascites (siehe oben) decapitierten Tiere enthielten 8–17 g hellgelben unblutigen Ascites. In Leber und (der sehr kleinen) Milz wurden Anzeichen von Vergiftung (weisser Überzug der Organe) beobachtet. Die Tumorbildung war stets gering und verzögert. Die Tumorhemmung scheint durch die Kombination des organischen Selenates mit Selenit hervorgerufen zu sein. Die angewandte Menge Na-Selenit (0,6 mg) war allein ohne Wirkung.

Arsensäure in organischer Bindung

Eine grosse Zahl organischer Arsensäure-Derivate sind in Zusammenhang mit P. EHRLICH's berühmten chemotherapeutischen Untersuchungen bekannt geworden. Hier beschreiben wir einige Phenyl-arsinsäuren, die wir einerseits zu Keimungsversuchen, andererseits zu Injektionen in tumortragende Ratten dargestellt haben.

Darstellung von p-Amino-phenyl-arsinsäure (Arsanilsäure, Atoxyl)

Ausgangsmaterial ist die p-Nitro-Phenylarsinsäure, die nach dem von JACOBS, HEIDELBERGER u. ROLF¹ angegebenen Prinzip gewonnen wurde.

p-Nitranilin	5,5 g	NaOH 10 % ig	92,0 ml
NaNO ₂	2,9 g in 5 ml H ₂ O	As ₂ O ₃ , 6,1 g gelöst in 31 ml 2-n. NaOH	

Das p-Nitranilin, in warmer 10 %iger HCl-Lösung gelöst, wurde bei 10° mit der Nitrit-Lösung diazotiert. Nach 15 Min. wurde die abgekühlte Diazoniumsalzlös. mit der 10 %igen kalten Natronlauge und unmittelbar darauf mit der Arsenit-Lösung versetzt. Die Mischung wurde auf dem Wasserbad bis zum Aufhören der Gasentwicklung erwärmt, angesäuert, mit aktiver Kohle versetzt und filtriert. Das im Vacuum eingeeengte Filtrat schied nach Ansäuern die p-Nitro-phenylarsinsäure in Form gelblicher Kristalle aus, die mit Eiswasser gewaschen wurden.

Zur Reduktion dieser p-Nitro-Verbindung wurden 6,5 g derselben, in Anlehnung an JACOBS und HEIDELBERGER sowie an BARTH (Ann. 429; 1922), in 100 ml 20 %iger Natronlauge gelöst und mit 40 g FeCl₂·4 H₂O, gelöst in 300 ml Wasser, versetzt. Dabei wurde das anfänglich grüne Hydroxyd durch Oxydation sehr schnell schwarz-braun. Das Ganze wurde auf 70° erwärmt² und durch einen weiten Büchner-Trichter filtriert. Durch Einengen des gelblichen Filtrats fiel zuerst reichlich Kochsalz und hierauf nach Ansäuern auf Kongoreaktion die p-Amino-arsenylsäure aus.

¹ W. A. JACOBS, M. HEIDELBERGER u. Ida. P. ROLF, J. Am. Chem. Soc. 40, 1580, 1918.

² Falls die Lösung nicht mehr stark alkalisch ist, muss zuerst 20 %ige Natronlauge zugegeben werden, damit nicht die gebildete Arsanilsäure als schwerlösliches Eisensalz in der Fällung zurückbleibt.

Umsetzung der Arsanilsäure mit Triose-Redukton

500 mg p-Amino-phenylarsinsäure, in 10 ml Wasser gelöst, wurden mit einer Lösung von 220 mg Triose-Redukton in 5 ml Wasser + 1 ml Eisessig versetzt. Nach etwa 1 Minute begann das Reaktionsprodukt in Form gelber Stäbchen auszukristallisieren. Nach 18stündigen Stehen bei 20° wurden die Kristalle abfiltriert, mit etwas Wasser gewaschen und getrocknet. Ausbeute an Rohprodukt 500 mg. Über 310° tritt (ohne Schmelzen) Verkohlung ein. Die Sbst. (Nr. HH 236) ist leicht löslich in verd. Alkali, schwer löslich in kaltem, wenig löslich in warmem Wasser, schwer löslich in Alkohol.

Äquivalent-Redukton-Bestimmung. 16,0 mg Sbst. + 10 ml Wasser mit 0,01-n Tillmans-Reagens (TR) unter Zusatz von etwas NaAc titriert, verbrauchten 13,5 ml TR.-Lös.

$$E_{\text{gef}} = \frac{16.0}{13.55 \times 0.01} = 118 \quad E_{\text{ber}} \text{ für } \text{C}_9\text{H}_{10}\text{O}_5 \text{ N As} = 144.$$

Umsetzung von Atoxyl mit Chlorameisensäure-äthylester

1 ml Pyridin wurde tropfenweise mit 0,5 ml Chlorameisensäure-äthyl-ester und hierauf mit 0,5 g Atoxyl versetzt und zum Sieden erhitzt, wodurch eine homogene Lösung entstand. Dieselbe wurde nach dem Erkalten dreimal mit Benzol extrahiert, um das überschüssige Pyridin möglichst vollständig zu entfernen.

Der hellbraune schmierige Rückstand ging auf Zusatz von 5 ml Wasser in Lösung und wurde dann mit HCl kongosauer gemacht. Innerhalb eines Tages kristallisierte das Reaktionsprodukt in Form hellbrauner Nadelchen und Stäbchen aus, die abfiltriert und mit Wasser gewaschen und getrocknet wurden. Ausbeute 200 mg Rohprodukt, das sich beim Erhitzen über 300° schwärzt.

Umkristallisieren aus wenig Alkohol in der Wärme unter Zusatz von Kohle. Nach Abfiltrieren von der Kohle und von etwas ungelöster Sbst. wurde das Filtrat stark eingengt und lieferte dann kleine gefärbte Kristallstäbchen. Reagiert nicht mit Triose-Redukton, was anzeigt, dass die Aminogruppe durch den Ameisensäure-ester blockiert ist. Sbst. HH 237 ist ziemlich leicht löslich in siedendem Wasser, leicht löslich in Methanol, Äthanol, verd. Alkali, schwer löslich in Benzol, Petroleumäther und Aceton.

Darstellung von 2-Amino-3-nitro-toluol und 2-Amino-5-nitro-toluol

Abweichend von GABRIEL und THIEME¹ (1919) sind wir von o-Toluidin (10 ml) ausgegangen, das mit 7 ml Eisessig und 20 ml Essigsäure-anhydrid acetyliert wurde. Nach Abkühlen auf 12° wurde die Lösung mit kleinen Portionen einer auf die gleiche Temperatur abgekühlten Salpetersäure-Essigsäure-Lösung (4,4 ml Salpeters. 1,52 in 6,5 ml Eisessig) unter Vermeidung eines Temperaturanstiegs über 20° versetzt. Nach Zusatz der ganzen Nitrierungs-Mischung verlief die Reaktion 30 Min. bei 12° und dann 18 Stunden bei 18° und lieferte dann eine feste Kristallmasse, die mit dem 3fachen Volumen Wasser verdünnt wurde. Die abfiltrierten, gewaschenen und getrockneten Kristalle (Ausbeute 8–9 g) bestanden aus einer Mischung von

2-Acetamino-3-nitro-toluol und
2-Acetamino-5-nitro-toluol.

¹ GABRIEL u. J. THIEME, Ber. d. d. chem. Ges. 52, 1080; 1919.

Diese Mischung wurde nicht fraktioniert sondern direkt deacetyliert. Zu diesem Zweck wurden 8 g der Kristallmischung unter Rückfluss mit 24 ml 20 % iger Salzsäure 2 Stunden gekocht, wobei die Kristalle allmählich in Lösung gingen, während ein gelbrotes Öl ausfiel.

Nach Abschluss der Reaktion wurde durch Zusatz der 3fachen Wassermenge das Öl zum Kristallisieren gebracht. Die dunkelgelbe Kristallmasse ergab, abfiltriert, gewaschen und getrocknet 5 g Rohprodukt von 2-Amino-3-nitro-toluol, Schmp 97° .

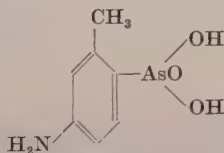
Das Filtrat dieser Fraktion wurde mit Ammoniak versetzt, worauf das 2-Amino-5-nitro-toluol in Form kleiner schmutziggelber Kristalle ausfiel, die nach Waschen und Trocknen etwa 1 g vom Schmp 128° ausmachten.

Darstellung der 2-Methyl-6-nitro-phenylarsinsäure

Das pulverisierte 2-Amino-3-nitro-toluol wurde in 25 ml 20 % iger Salzsäure gelöst, und mit einer Lösung von 2,75 g Natriumnitrit bei 10° diazotiert. Die Diazoniumlösung wurde unter ständigen Umrühren in die auf 0° abgekühlten 90 ml 10 % ige Natronlauge gegossen; unmittelbar darauf wurde die Arsenitlösung (5,1 g As_2O_3 in 26 ml 2-n. Natronlauge) zugesetzt und die Mischung bei $60\text{--}70^{\circ}$ 2 Stunden lang erwärmt. Nach Ansäuern mit Essigsäure wurde filtriert und das Filtrat im Vacuum eingengt. Mit konz. Salzsäure wurde kongosaure Reaktion eingestellt, worauf 2-Methyl-6-nitro-phenylarsinsäure in Form eines hellgelben Kristallpulvers ausfiel. Ausbeute 2 g von Schmp. $228\text{--}230^{\circ}$.

Darstellung der 2-Methyl-4-amino-phenylarsinsäure (500)

Die 2-Methyl-4-nitro-phenylarsinsäure (1,5 g) wurde in 30 ml 20 % iger Natronlauge gelöst, worauf eine warme Lösung von Ferrochlorid unter Umrühren zugesetzt wurde. Das sofort ausgefallte Ferrohydroxyd färbte sich schnell schwarzbraun. Nach 15 Min. wurde das Hydroxyd abfiltriert und das Filtrat eingengt. Auskristallisierendes Kochsalz wurde durch Filtrieren entfernt, das Filtrat kongosauer gemacht, worauf die Amino-phenylarsinsäure in Form eines schwach bräunlichen Pulvers auskristallisierte. Die Ausbeute an der gewaschenen und getrockneten Subst. betrug 1,2 g vom Schmp 224° .



Umsetzung der p-Amino-phenylarsinsäure mit p-Chinon (239)

Unter den tumorhemmenden Stoffen haben sich Chinone oft besonders wirksam erwiesen, wie besonders Versuche von DOMAGK und von HACKMANN gezeigt haben. Wir haben aus diesem Grund die p-Amino-phenylarsinsäure mit p-Chinon in folgender Weise kombiniert.

Mit 1 g p-Chinon in 100 ml Wasser gelöst wurde eine Lösung von 1 g Atoxyl in 400 ml Wasser gemischt. Die Lösung, die sich während 1 Tages bei Zimmertemperatur stark dunkelbraun färbte, wurde im Vacuum stark eingengt, worauf kleine dunkelbraune Kristalle ausschieden. Sie wurden abfiltriert, gewaschen und getrocknet und bildeten ein Rohprodukt von 400 mg, das bis 310° nicht schmolz.

Die Subst. erwies sich in allen üblichen Lösungsmitteln als schwer löslich; sie wurde umkristallisiert durch Auflösung in verd. Alkali und Ansäuern mit Essigsäure, wodurch sie langsam in der oben erwähnten Form ausfiel.

Aus dem Filtrat der ersten Kristallisation des Rohproduktes wurden bei weiterem Eindunsten hellere Kristalle erhalten.

Keimungsversuche mit Arsanil-Derivaten an Gerstensamen

Die Gerstensamen wurden nach unserer früheren Methodik (Arkiv 20 A nr 2) zuerst 8–10 Stunden in Wasser und dann in der Lösung L des zu untersuchenden Stoffes gequollen. Nach 15–20 Stunden wurden sie in flachen Schalen zwischen Filtrierpapier ausgebreitet, das mit der Lösung L beschickt war. Dieselben wurden stets auf pH 6,0–6,5 gehalten. Stets Kontrollversuche mit Wasser bei exakt dem gleichen pH.

Arsanilsäure = 2-Amino-phenyl-arsinsäure

Konzentration der Lösung L: 3 mg in 100 ml auf 3 g Samen.

Etwa 15 % der Samen keimen vom 3. Tag an aus. Nach 10 Tagen entwickeln sich die Keimblätter normal. Nach 14 Tagen 70 mm lang, Wurzeln bleiben kurz (3–5 mm). Wurzeln im Wasserkontrollversuch 20–30 mm.

Redukton-Arsanilsäure (HH 236)

Konzentration der Lösung: 3 mg in 100 ml auf 3 g Samen.

Innerhalb 5 Tagen beginnt etwa die Hälfte aller Samen zu keimen. Keimblätter nach 10 Tagen 50–70 mm, kräftig ausgebildet, breit. Kurze Wurzeln (3–5 mm). Wasserkontrolle: Keimblätter: 120 mm. Länge, normale Wurzeln.

Arsanilsäure-Chlorameisensäure (HH 237)

Konzentration der Lösung L: 10 mg in 100 ml.

Innerhalb 7 Tagen etwa die Hälfte der Samen gekeimt, Keimblätter 10–15 mm. Wasserkontrolle Keimblätter 10–20 mm. Also geringer Einfluss auf die Keimfähigkeit.

Redukton-2-Amino-6-Methyl-phenylarsinsäure

Der Versuch mit HH 237 wurde in der Weise wiederholt, dass die verwendeten Samen (Versuche und Wasser-Kontrollen) während der Vorbehandlung 2 Stunden a) mit ultravioletttem Licht, b) in besonderem Versuch mit Röntgenstrahlen bestrahlt wurden.¹ Die UV-Bestrahlung hatte wenig Einflüsse auf die Keimung; auch nach dieser Bestrahlung war die Keimungs erniedrigung unbedeutend.

Dagegen wurde durch die vorhergehende Röntgen-Bestrahlung die Keimungs-erniedrigung durch den Arsanilsäure-Ameisensäure-ester wesentlich verstärkt. Wir werden auf diesen Einfluss mit weiteren Versuchen unter Variation der Röntgendosen zurückkommen.

¹ Wir danken auch hier Herrn Laborator Dr. ARNE FORSSBERG für die freundliche Hilfe im Radiophysikalischen Institut (Prof. E. SIEVERT) des K. Karolinischen Institutes.

Redukton-2-Amino-6-Methyl-phenyl-arsinsäure

Konzentration der Lösung L: 6 mg in 100 ml auf 5 g Samen.

Innerhalb von 8 Tagen zeigte sich kein wesentlicher Unterschied in der Keimung gegenüber den Wasserkontrollen, abgesehen von einer Verkürzung der Wurzeln, die aber auffallenderweise die Entwicklung der Pflanze fast gar nicht beeinflusste.

Arsanilsäure-p-Chinon

Diese Verbindung ist so schwer löslich in Wasser, dass sie bei den Tierversuchen nur in Suspension angewandt werden konnte. Eine solche Suspension (5 mg in 100 ml), die übrigens wenig stabil ist, unterdrückte die Keimung der Gerstensamen vollständig. Es gelang auch nicht, mit viel geringeren Mengen der Arsanils.-Chinon Suspension Chlorophylldefekte hervorzurufen.

In enzymatisch wirksamen Homogenaten keimender Gerste wird unser Arsanils.-Chinon schnell reduziert und zugleich gespalten, was die Verfolgung der Reduktionswirkung erschwert.

Wir haben analoge Versuche mit keimenden Bohnen angestellt und Homogenate der isolierten Embryonen zur Wirkung gebracht. Sie haben sich — je nach dem Stadium der Samenkeimung — mehr oder weniger enzymatisch hochaktiv erwiesen. An der deutlich nachweisbaren Bildung von Brenzcatechin scheinen Ascorbinsäure und Glutathion teilzunehmen.¹

Am Stoffwechsel in diesen Homogenaten sind ferner Dehydrasen beteiligt. Besonders kompliziert wird der Mechanismus des Chinon-Umsatzes durch die Anwesenheit von Cu, das als Katalysator wirkt und vermutlich nach CHARGAFF auch als Antagonist des Glutathions.

Versuche mit Yoshida-Ascites-Sarkom-Ratten

Methodik: Intraperitoneale Injektion der Lösung der zu prüfenden Substanz (physiolog. Kochsalzlös., pH 7,3–7,7) nach der Injektion des Ascites (am 1. Tag in unmittelbarer Folge).

Rattengewichte: Die Angaben beziehen sich auf Anfang und Ende des Versuches.

Atoxyl = 2-Amino-phenyl-arsinsäure

Dosis: 4 Injektionen zu je 3 mg.

Ergebnisse: Lebensdauer nach Injektion 4–5 Tage, gleich derjenigen der Kontrollratten. Reichliche Tumorbildung. Tumoren stark blutgefüllt. Leber und Milz meist normal.

Arsanilsäure-Redukton (vgl. S. 586)

Gewicht der Ratten: 160–190 g.

Dosis: 5 Injektionen zu je 3 mg = 15 mg.

Ergebnisse: Lebensdauer nach Injektion 1 Tag verlängert. Ascites im Mittel 12 g, wenig blutig, wie auch im Kontrollversuch. Tumorbildung weniger reichlich als in den Kontrollversuchen.

Leber 10–12, Milz 1,1–1,25: wie in den Kontrollversuchen.

¹ Vgl. EULER und HASSELQUIST, dieses Arkiv 9, im Druck. — EULER, dieses Arkiv 1, nr 56, 1949.

Arsanilsäure-Chlorameisensäure (HH 237)

Gewicht der Ratten: 200–230 g.

Dosis: 4 Injektionen zu je 1,5 mg = 6 mg.

Ergebnisse: Lebensdauer 5 Tage, nur wenige Stunden länger als die der Kontrollratten. Ascites reichlich, stark blutig. Reichliche Tumorbildung. Leber 13–15 g, normal. Milz 1 g normal, wie die der Kontrollratten.

2-Nitro-6-methyl-arsenylsäure

Gewicht der Ratten: 137–174 g.

Dosis: 5 Injektionen zu je 3 mg oder 2 mg = 14 mg.

Ergebnisse: Lebensdauer 1–2 Tage grösser als die der Kontrollratten. Ascites (fast unblutig), im Mittel 13 g gegenüber 20 g der Kontrollratten. Reichliche Tumorbildung. Lebern 9–11,6 g, durch den Tumor mit Magen und Darm zusammengewachsen. Milz 0,6–1,3 g. Der ein paar Tage höheren Lebensdauer zufolge sind die festen Tumoren der Versuchstiere noch reichlicher entwickelt als die der Kontrolltiere.

Redukton-2-Amino-6 Methyl-phenylarsinsäure

Gewicht der Ratten: 180–200 g.

Dosis: 4 Injektionen zu je 3 mg oder 2 mg = 11 mg.

Ergebnisse. Lebensdauer nicht erhöht. Ascites-Menge variierend. Tumorbildung reichlich, nicht geringer als bei den Kontrollratten. Leber der Versuchstiere 10–12 g, Oberfläche angegriffen.

Milz 0,4–0,5 g, also sehr klein. Der Magen voll von einer harten Masse, während der Darm fast leer war. Mehrere Versuchstiere haben während des Versuchs an Gewicht *verloren*, obwohl Ascites und Tumor sich entwickelten. Anscheinend hat die injizierte Substanz die Bewegungen des Magens und Darmes gelähmt.

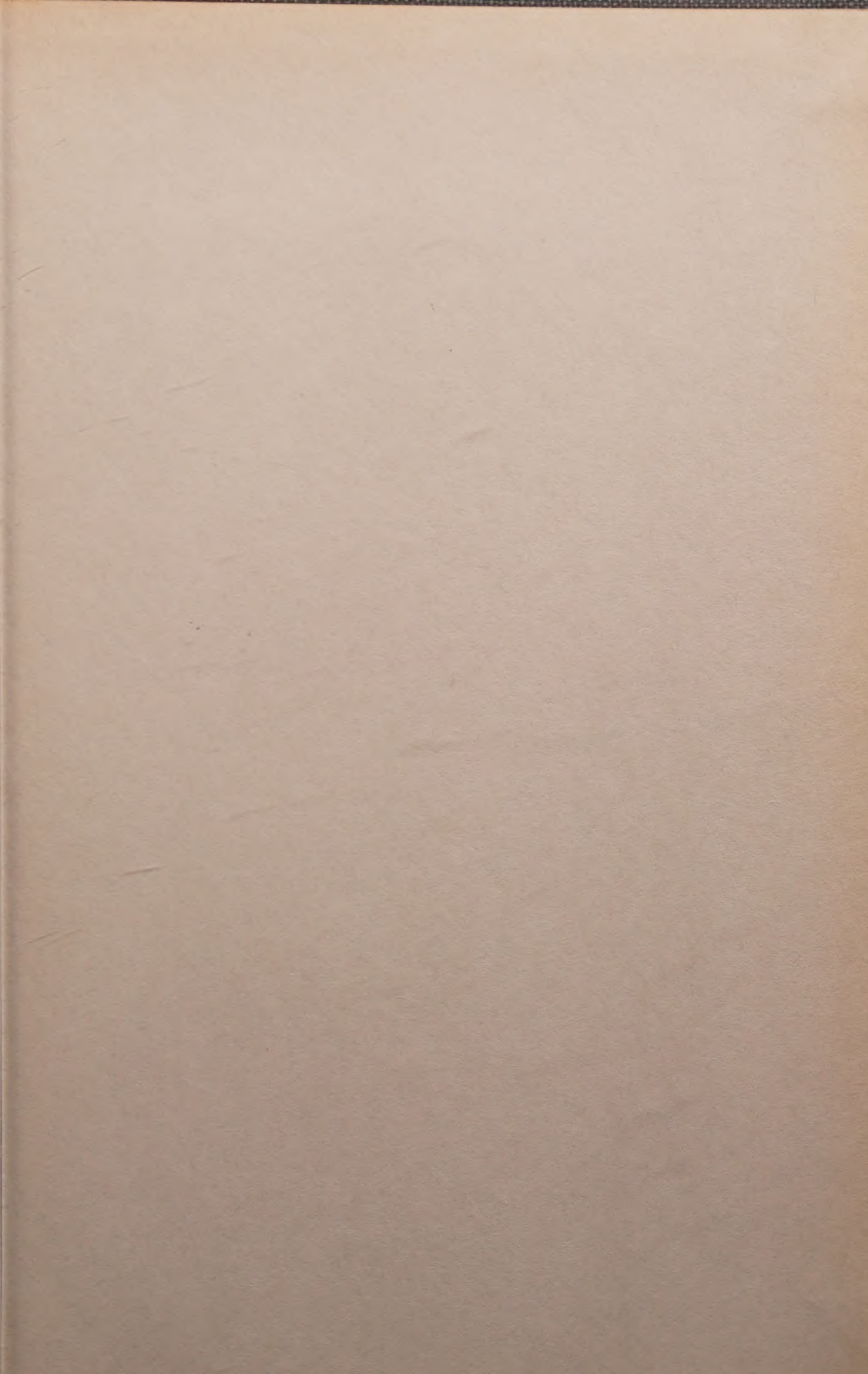
Herrn Ingenieur H. WÄHLSTAM danken wir für eifrige Mitarbeit.

Stockholm, Universität, Vitamin-Institut u. Inst. für organ.-chem. Forschung.

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